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# Plum Curculio Control Experiments in 1955–58[1]

OLIVER I. SNAPP, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

ABSTRACT

Cage tests showed the good residual value of Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) and Sevin® (1-naphthyl *N*-methyl carbamate) and good short-lived effectiveness of Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) against the plum curculio (*Conotrachelus nenuphar* (Hbst.)) on peaches. In field tests the recommended regular schedule of five applications of 2 pounds of 15% parathion, wettable powder per 100 gallons controlled light to moderate infestations. Most effective was a split schedule of three applications of dieldrin, 1 pound of wettable powder containing 50% of the active ingredients, followed by two of parathion, 2 pounds of wettable powder containing 15% of the active ingredient, per 100 gallons. Guthion was about as effective as parathion, and Sevin and Perthane® (1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane) were promising. Malathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), and heptachlor were less effective than parathion, and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate), aldrin, and Strobane® (a mixture of chlorinated terpenes with about 66% chlorine) were less effective. Dieldrin at ¼ pound per 100 gallons was less effective than at 1 pound, but at either strength it should not be used later than 2 weeks after the shuck-off application. Harvest residues of Guthion and Sevin were well below the approved tolerances on peaches, and those for Perthane were 1.3 to 2.1 p.p.m. Lime did not interfere with the effectiveness of parathion and malathion. The petal-fall application was found of value.

New organic insecticides have been investigated for control of plum curculio (*Conotrachelus nenuphar* (Hbst.)) on peaches at Fort Valley, Georgia, for many years. This paper reports on cage tests and field experiments conducted during the period 1955–58.

CAGE TESTS.—New insecticides were given preliminary evaluation by methods previously described (Snapp 1947).

In 1955 as little as 4 ounces of Guthion® (*O,O* dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), wettable power containing 50% of the active ingredient, per 100 gallons displayed very good knockdown of overwintering adults and quick kill. It gave 100% mortality within 2 days after it had weathered for 3 days before the insects were exposed to it. It was equally effective against new first-generation adults, though they are usually more resistant to insecticides than those that overwinter. The residual value of Guthion was equally good. After weathering for 10 days before beetles were exposed to them, the residues from 4 ounces of the wettable powder killed all new first-generation adults within 4 days and those from 8 ounces gave complete kill within 1 day. Seventeen-day-old residues from the 8-ounce dosage gave 100% kill within 14 days, and 42-day-old residues from a 12-ounce dosage within 10 days.

In similar tests in 1955 fresh deposits of Diazinon® (*O,O*-diethyl *O*-(2-isoropyl-4-methyl-6-pyrimidinyl) phosphorothioate) gave good to very good control and those of Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) fairly good. The residual value of Diazinon was less than that of Guthion against both overwintered and new first-generation adults, and that of Dipterex was very poor.

In 1957 Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) displayed good knockdown and effectiveness when used at 2 pounds of wettable powder containing 25% of the active ingredient per 100 gallons. It killed 40% of new first-generation adults and, caused 40% to be abnormal within 1 day, and killed 90% within 2 days and 100% within 3 days. It displayed rather poor knockdown when used at half that rate. The residual value of Trithion was poor. At the 2-pound dosage 5-day-old residues killed only 10% of new first-generation adults within 1 day, 30% within 2 days, 50% within 3 days, and 60% within 18 days.

Fresh deposits of Sevin® (1-naphthyl *N*-methyl carbamate), 2 pounds of wettable powder containing 50% of the active ingredient, per 100 gallons, were slow-acting, killing only 20% of new first-generation adults within 1 day, 50% within 2 days, 70% within 4 days, 90% within 7 days, and 100% within 9 days. Of those not killed earlier, 30% were abnormal on the second day, 10% on the third day, and 20% on the fourth day. The value of moderately aged residues was good and of rather old residues poor. Nine-day-old residues from the 2-pound dosage killed 80% of the adults exposed to them within 5 days and 100% within 7 days. Sixteen-day-old residues gave no kill in 6 days and only 20% in 12 days.

[1] Accepted for publication September 16, 1959. Presented at the Sixth Annual Meeting of the Entomological Society of America at Salt Lake City, Utah, December 1–4, 1958.

[2] Julius P. Hollon assisted with the experiments reported in this paper.

FIELD EXPERIMENTS.—The experiments were conducted by methods outlined in earlier papers (Snapp 1947, 1952). All applications were made with a high-pressure hydraulic sprayer. The following schedules were used:

A. Petal-fall, shuck-off, 2 weeks after shuck-off, and 4 and 2 weeks prior to harvest.
B. Shuck-off, 2 weeks after shuck-off, and 4 and 2 weeks prior to harvest.
C. One insecticide at petal fall, shuck-off, and 2 weeks after shuck-off, and a second insecticide 4 and 2 weeks prior to harvest.
D. Petal fall, shuck-off, 2 weeks after shuck-off, and 4 weeks prior to harvest.

These experiments were replicated in a randomized set-up of single-tree plots. In 1956 and 1957 each tree was artificially infested with 12 overwintered plum curculio adults in March or April, and later by placing 25 mature first-generation larvae on the ground under each tree. In 1958 one Sullivan's Elberta and two Regular Elberta trees were similarly infested.

*1956 Experiments.*—In 1956 there were two replications in a Regular Elberta peach orchard. The materials tested and the results are shown in table 1.

Since the crop in the test orchard was light and irregular and the number of replications limited, only wide differences in control in dropped and harvested fruit are significant. Under heavy infestations, as revealed by the 44.2% of infested fruit from the untreated trees at harvest, parathion alone or with hydrated lime, dieldrin alone or in a split schedule with parathion, Guthion and Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane) gave excellent control. Parathion was less effective when the petal-fall application was omitted (schedule B) and dieldrin when only ½ pound of the wettable powder was used. Malathion, Dipterex, aldrin, and heptachlor were slightly less effective than the materials giving excellent control; and malathion, Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O*,*O*-dimethyl phosphorothioate), Diazinon, and Strobane® (a mixture of chlorinated terpenes with about 66% chlorine) gave poor control. As with parathion, hydrated lime did not impair the effectiveness of malathion. None of the treatments caused any noticeable injury.

**Table 1.—Results of orchard experiments for the control of the plum curculio on Regular Elberta peaches, season of 1956.**

| Insecticide (Pounds of Wettable Powder per 100 Gallons) | No. of Applications | Schedule | Per Cent of Drops Infested | Per Cent of Fruit Infested at Harvest |
|---|---|---|---|---|
| Parathion (15%) 2 | 5 | A | 4.0 | 0.6 |
| | 4 | B | 14.8 | 3.1 |
| 2+hydrated lime 8 | 5 | A | 9.5 | .2 |
| Malathion (25%) 4 | 5 | A | 7.3 | 7.4 |
| 4+hydrated lime 8 | 5 | A | 11.4 | 4.0 |
| Dipterex (50%) 0.5 | 5 | A | 1.2 | 4.0 |
| Guthion (50%) 0.5 | 5 | A | 2.9 | 1.4 |
| Chlorthion (25%) 2 | 5 | A | 4.3 | 7.9 |
| Diazinon (25%) 2 | 5 | A | 57.6 | 10.8 |
| Dieldrin (50%) 0.5 | 4 | D | 4.0 | 7.3 |
| 1 | 4 | D | 0 | 0.9 |
| 0.5+parathion (15%) 2 | 3+2 | C | 1.2 | 0.3 |
| 1+parathion (15%) 2 | 3+2 | C | 3.1 | 0.1 |
| Aldrin (25%) 2 | 4 | D | 7.5 | 6.1 |
| Heptachlor (25%) 2 | 4 | D | 18.4 | 4.8 |
| Strobane (50%) 2 | 4 | D | 27.4 | 25.8 |
| Perthane (50%) 2+dispersing agent 1% | 4 | D | 6.0 | 1.7 |
| Untreated | — | — | 21.8 | 44.2 |

**Table 2.—Results of orchard experiments for the control of the plum curculio on Sullivan's Elberta peaches, season of 1957.**

| Insecticide (Pounds of Wettable Powder per 100 Gallons) | No. of Applications | Schedule | Infested Fruit at Harvest (%) |
|---|---|---|---|
| Parathion (15%) 2 | 5 | A | 26.5 |
| 2+hydrated lime 8 | 5 | A | 11.8 |
| Guthion (15%) 1.5 | 5 | A | 19.2 |
| Dieldrin (50%) 0.5 | 4 | B | 9.9 |
| 0.5+parathion (15%) 2 | 3+2 | C | 16.7 |
| 1+parathion (15%) 2 | 3+2 | C | 0 |
| Perthane (50%) 2 | 4 | B | 30.0 |
| 2+dispersing agent 1% | 4 | B | 37.9 |
| Untreated | — | — | 43.1 |

*1957 Experiments.*—These experiments were replicated eight times in a Sullivan's Elberta peach orchard. The insecticides and schedules used and the results at harvest from two trees per treatment are given in table 2.

As in 1956, the crop was light and irregular, the number of replications with a suitable crop for examination at harvest limited, and the infestation heavy, 43.1% of the fruit on the untreated trees being wormy at harvest. Under these conditions treatments were subjected to a severe test and only a dieldrin-parathion split schedule gave good control, and then only when the dieldrin was used at 1 pound of the wettable powder per 100 gallons. When the dieldrin dosage was reduced, control was poor as it was with parathion and Guthion, which were about equally effective. Again hydrated lime did not impair the effectiveness of parathion; in fact, it may have had some beneficial effect. Dieldrin at the ½-pound dosage in a complete schedule was second only to the better dieldrin-parathion split schedule, giving fairly good control. Perthane alone or with a dispersing agent gave disappointingly poor control.

None of the treatments affected the flavor or injured the fruit or any part of the trees.

*1958 Experiments.*—These experiments were set up in a peach orchard containing two varieties—two replications on 6-year-old Sullivan's Elberta and eight on 3-year-old Regular Elberta trees. The insecticides and schedules used and the results are given in table 3.

The percentages of dropped and harvested fruit damaged by the plum curculio on the untreated trees showed a heavy early-season and light late-season attack. Early in the season only dieldrin and the higher dosage of Perthane showed promise. However, on the basis of the results at harvest, Guthion at 1¼ pounds and Sevin at 2 pounds were considered highly promising. As previously, a split schedule of dieldrin and parathion was outstanding.

None of the treatments affected the flavor or injured the fruits or any part of the trees.

HARVEST RESIDUES.—Chemical analyses of the resi-

**Table 3.—Results of orchard experiments for the control of the plum curculio, season of 1958.**

| INSECTICIDE (POUNDS OF WETTABLE POWDER PER 100 GALLONS) | NO. OF APPLICATIONS | SCHEDULE | PER CENT OF DROPS INFESTED | PER CENT OF FRUIT INFESTED AT HARVEST | |
|---|---|---|---|---|---|
| | | | SULLIVAN'S ELBERTA | Sullivan's Elberta | Regular Elberta |
| Parathion (15%) 2 | 5 | A | 45.0 | 1.9 | 2.2 |
| Guthion (25%) 0.75 | 5 | A | 32.5 | 0.7 | 3.2 |
| 1.25 | 5 | A | 47.5 | 0 | 0.4 |
| Dieldrin (50%) 1 +parathion (15%) 2 | 5 | C | 0 | 0 | 0.4 |
| Perthane (50%) 4 | 4 | B | 5.9 | 0 | 1.3 |
| 2 | 5 | A | 37.5 | 1.9 | 3.1 |
| Sevin (50%) 2 | 5 | A | 58.8 | 0.9 | 0.0 |
| Untreated | — | — | 75.0 | 0.8 | 7.6 |

dues at harvest on peaches from several plots are shown in table 4.[3] Only materials have been included for which adequate residue data were not known to be available at the time the samples were taken.

The amounts of Guthion, Sevin, and Diazinon were well below those permitted by the tolerances covering their use on peaches. On the other hand, the data show that dieldrin cannot be used at ½ pound or more of the wettable powder in a full spray schedule without danger of exceeding the tolerance of 0.1 p.p.m. However, it can be used safely up to at least 1 pound through the 2 weeks after shuck-off application. Limited data indicate that use of heptachlor at the 2-pound dosage should also be confined to early applications. In a complete program the harvest residue was considerably in excess of the tolerance. Tolerances have not been established for Strobane, Perthane, Dipterex, and Chlorthion on peaches.

**Table 4.—Insecticide residues on peaches at harvest.**

| INSECTICIDE | POUNDS OF WETTABLE POWDER PER 100 GALLONS | NO. OF APPLICATIONS | DATE OF LAST APPLICATION | RESIDUE (P.P.M.) |
|---|---|---|---|---|
| | *1956 Experiments* | | | *July 10* |
| Guthion (50%) | 0.5 | 5 | June 29 | 0.12 |
| Dieldrin (50%) | 0.5 | 3 | April 18 | .02 |
| | 1 | 3 | Do. | .05 |
| | 0.5 | 4 | June 12 | .07 |
| | 1 | 4 | Do | .32 |
| Aldrin (25%) | 2 | 4 | Do | .05 |
| Strobane (50%) | 2 | 4 | Do | .92 |
| Perthane (50%) +dispersing agent 1% | 2 | 4 | Do | .82 |
| Dipterex (50%) | 0.5 | 5 | June 29 | .02 |
| Chlorthion (25%) | 2 | 5 | Do | 0 |
| Diazinon (25%) | 2 | 5 | Do | 0 |
| | *1957 Experiments* | | | *July 1* |
| Guthion (15%) | 1.5 | 5 | June 17 | 0.08 |
| Dieldrin (50%) | 0.5 | 3 | May 5 | 0 |
| | 1 | 3 | May 8 | .09 |
| | 0.5 | 4 | June 3 | .13 |
| Perthane (50%) | 2 | 4 | Do | 1.34 |
| | *1958 Experiments* | | | July 22 |
| Guthion (25%) | 0.75 | 5 | July 8 | 0 |
| | 1.25 | 5 | Do | 0 |
| Dieldrin (50%) | 1 | 3 | May 6 | .01 |
| Perthane (50%) | 4 | 4 | June 25 | 2.10 |
| | 2 | 5 | July 8 | 1.60 |
| Sevin (50%) | 2 | 5 | Do | .14 |

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**Snapp, Oliver I.** 1952. Plum curculio control on peach in 1951. Jour. Econ. Ent. **45**(2): 245–51.

[3] The analyses were made by the Pesticide Chemicals Research Branch.

# Reaction of Drosophila to Different Varieties of Tomatoes[1]

HORATIO C. MASON, THOMAS J. HENNEBERRY, and RICHARD LEHR[2,3] *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

ABSTRACT

Studies were conducted at Beltsville, Maryland, to determine whether varieties of tomatoes differ in attractiveness to *Drosophila melanogaster* Meig. adults. San Marzano and Pennheart were the least attractive and Rutgers was the most attractive. Other varieties compared were Grothen's Globe, Pritchard, Burpee Hybrid, Pan America, Greater Baltimore, Pearson, Garden State, Marglobe, Burpee Globe, Chesapeake, Stokesdale, and John Baer.

It is general knowledge among commercial growers and processors of tomatoes that some varieties are more subject to cracking than others. Severe cracking of the fruit in the field is favorable for drosophila development (Michelbacher *et al.* 1953). Tomatoes that are cracked during harvest or in transit to the processing plant are also attractive to these flies (Miller 1956), and by the time they are processed individual tomatoes may be infested with several hundred eggs and larvae.

In 1954 investigations were undertaken at Beltsville, Maryland, to determine whether varieties of tomatoes differed in attractiveness to adults of *Drosophila melanogaster* Meig. U. S. No. 1 or No. 2 field-grown canning-grade tomatoes were placed at random on a turntable 5 feet in diameter within 1 to 2 hours after harvest. With the table making about one revolution per minute, they were exposed to several thousand flies that had been released in a $15 \times 11\frac{1}{2} \times 9$ foot room. Bright sunlight was eliminated during the day by regulating the venetian blinds that covered the windows on two sides of the room.

TESTS IN 1954.—Twelve varieties of tomatoes were set in the field on June 3 and 4, each variety in a 1-row plot 24 feet long. The plants were spaced 4×4 feet and unstaked. The planting was in randomized blocks with four replications. A 5-10-5 fertilizer was applied twice during the season by side dressing. Overhead irrigation was used when needed.

Sixteen tests were run between August 25 and October

[1] Accepted for publication September 15, 1959.

[2] Richard Lehr resigned in 1957.

[3] B. K. Dozier, formerly of the Entomology Research Division, assisted with these studies in 1954.

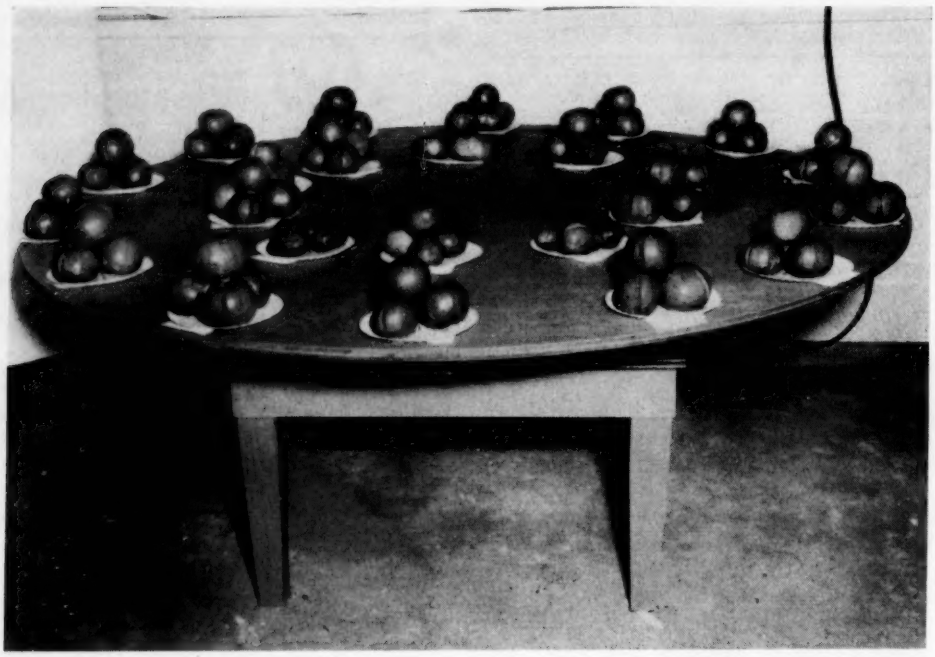

FIG. 1.—Technique used in 1955 and 1956 for studying the attractiveness of different varieties of tomatoes to drosophila adults.

4. For each test one ripe tomato was picked at random from each plot in the same block, a different block being used for each test. A pyramid-like section about 1½ inches on a side was cut from each tomato and placed in a pint fruit jar. A cone-shaped paper drinking cup with an opening in the tip just large enough for the flies to pass through was snugly fitted into the mouth of each jar with the tip down. The trap jars were then placed at random on the turntable and exposed to the drosophila flies that had been released in the room. After 24 hours the trapped flies were killed with carbon tetrachloride and counted.

The results are summarized in table 1. Fruits from San Marzano, Pennheart, Grothen's Globe, Pritchard, and Burpee Hybrid attracted fewer flies than Marglobe, Burpee Globe, and Rutgers. Garden State attracted significantly more flies than San Marzano and Pennheart. Pan America, Greater Baltimore, and Pearson did not differ significantly from any of the other varieties.

TESTS IN 1955.—Nine of the 12 varieties were the same as those tested in 1954. The plants were set in the field on June 8, each variety in a 1-row plot 18 feet long. The rows were 5 feet apart and the plants were spaced 3 feet in the rows. The varieties were completely randomized with five replications. Cultural practices were about the same as in 1954.

For each of the 4 tests run between September 5 and 22, 2-tomatoes were picked from each plot, and from the 10 tomatoes of each variety 2 samples of 4 were selected. Results were based on the number of eggs laid in a vertical slit about 1½ inches long cut in each tomato. Each sample of four fruits was placed on a 6-inch paper plate, and the plates were placed at random on the turntable (fig. 1). After exposure to the flies for about 18 hours, the plates were removed and the eggs in each slit were counted under a binocular stereoscopic microscope.

As shown in table 1, significantly fewer eggs were laid in fruit from San Marzano and Pennheart varieties than in Garden State, Pearson, John Baer, and Rutgers. Greater Baltimore differed from John Baer and Rutgers and Stokesdale differed from San Marzano. Pan America, Chesapeake, Pritchard, and Marglobe did not differ significantly from any of the others.

TESTS IN 1956.—Plants for the varieties compared in 1956 were transplanted to the field on June 5 and 6, each variety in plots 3 rows wide and 16 feet long. The

**Table 1.—Attractiveness of different varieties of tomatoes to *Drosophila melanogaster* as measured by flies trapped in jars or eggs laid in slit ripe tomatoes.**

| VARIETY | MEAN NUMBER OF ADULTS TRAPPED PER JAR PER TEST | MEAN NUMBER OF EGGS LAID PER PLATE PER TEST | |
|---|---|---|---|
| | 1954 | 1955 | 1956 |
| San Marzano | 351 | 349 | — |
| Pennheart | 352 | 372 | 445 |
| Grothen's Globe | 378 | — | — |
| Pritchard | 405 | 419 | — |
| Burpee Hybrid | 412 | — | — |
| Pan America | 465 | 418 | 715 |
| Greater Baltimore | 480 | 386 | — |
| Pearson | 486 | 529 | 716 |
| Garden State | 507 | 525 | 648 |
| Marglobe | 573 | 431 | 673 |
| Burpee Globe | 606 | — | — |
| Rutgers | 614 | 552 | 776 |
| Chesapeake | — | 420 | 781 |
| Stokesdale | — | 517 | — |
| John Baer | — | 540 | — |
| L.S.D. at 5% level | 150 | 147 | 130 |

rows were 4 feet apart and the plants were spaced 4 feet in the rows. The experimental design was randomized blocks with six replications. Cultural practices were the same as for the previous 2 years except that irrigation was not available. Precipitation at Beltsville from June 6 to September 11 was about 10.71 inches.

One ripe tomato was picked at random from each plot for each of the seven tests that were run between August 28 and September 11. The six tomatoes of each variety were mixed and two samples of three each were selected. After the tomatoes had been slit as in 1955, each sample was placed on a 6-inch paper plate. The plates were placed on the turntable and exposed to a heavy population of flies for about 16½ hours before the slits were examined for eggs.

The results are summarized in table 1. Pennheart was the least attractive variety. Fewer eggs were laid in Garden State tomatoes than in Chesapeake, but the former did not differ significantly from Marglobe, Pan America, Pearson, and Rutgers.

THREE-YEAR COMPARISON.—Of the six varieties compared in all three years, Pennheart was consistently less attractive than Garden State and Rutgers. Pan America, Pearson, Garden State, Marglobe, and Rutgers did not differ significantly in any year.

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# Feed Lot Tests with Ronnel for Control of Cattle Grubs[1]

HAROLD E. THURBER and GEORGE D. PETERSON, JR.,[2] *University of California Agricultural Extension Service, El Centro*

ABSTRACT

Ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate; formerly designated Dow ET-57 and manufactured under the trade name Trolene) was administered orally to steers and heifers in a commercial feed lot in the Imperial Valley, California for control of *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.). It was given in a bolus at the rate of 100 mg./kg. of body weight. Effective control of cattle grubs was obtained even in animals treated late following infestation. Best results were obtained when treatment was made before the grubs reached the animal's back. Hide damage and carcass trim were greatly reduced on the treated animals as compared with the controls. No significant weight gain of the treated animals over the controls was noted.

The use of the systemic insecticide ronnel (*O,O*,-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate); also known as Trolene, or Dow ET-57, for the control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.), has been reported by various workers. Roth and Eddy (1957) and McGregor and Bushland (1957) reported that oral administration of ronnel was effective against encysted grubs of both species as well as against the encystment of new ones. Jones & Brundrett (1957) and Adkins (1957) reported that ronnel was effective when given orally before the grubs encysted. Knapp *et al.* (1958) discussed results of field studies with feed and bolus formulations of ronnel against cattle grubs. More recently Harris *et al.* (1959) and Jones (1959) have reported on the effectiveness of this material for control of cattle grubs.

In tests conducted in a commercial feed lot in the Imperial Valley, California, during the winter of 1957–58, the authors found ronnel effective against the aforementioned cattle grubs in beef cattle when administered orally at 100 mg./kg. of body weight. The Imperial Valley is unique in that most of the beef cattle fed out in local feed lots are imported from other areas and become infested with cattle grubs at their points of origin. Cattle arriving at local feed lots may have grubs showing in the back as early as September. Both *H. lineatum* and *H. bovis* occur in these imported animals. Both species were present in the animals used in the experiments reported here.

PROCEDURE.—Steers and heifers numbering 114 were used in these trials. These animals comprised three different groups designated K-1, J-1, and BJ-1. K-1 animals were big-boned northern Hereford steers somewhat older than the other two groups and weighing approximately 750 pounds each. The J-1 group were southern crossbred steers averaging approximately 580 pounds and lacking the quality of the K-1 animals. The BJ-1 cattle comprised a group of southern heifers, predominantly Hereford, averaging about 510 pounds.

Each group of cattle was divided into treated and control lots. All animals were surveyed for grubs and weighed by lots on December 11, 1957. Each animal was ear tagged for identification purposes. Grubs were located by palpation, but their locations were not plotted. This procedure was repeated at intervals until slaughter. Treated animals were given a single treatment of ronnel in a bolus at the rate of 100 mg./kg. of body weight on December 11, 1957. Thus three trials were conducted concurrently. Each animal was identified at slaughter and the hide and carcass examined for encysted grubs and grub damage.

[1] Accepted for publication November 6, 1959.

[2] The ronnel used in these studies was furnished by Dow Chemical Co., Seal Beach, California. The authors also wish to acknowledge the cooperation of Jackson Feed Mill, Inc., El Centro, Anza Packing Company, El Centro, Union Packing Company, Los Angeles, and Dr. G. K. L. Knott, Imperial County Veterinarian. Appreciation is expressed to Dr. P. C. Enge, University of California Agricultural Extension Service, Davis, for assistance in planning and evaluating these studies.

**Table 1.—Comparison of average cattle grub counts and daily gain in beef cattle treated with ronnel in Imperial Valley, California.**

| No. Head | Treatment | Average Days to Slaughter | Initial Grub Count | Periodic Grub Count[a] (Days from Treatment) | | | | | Average Weight per Head (Lb.) | | Average Daily Gain for Period (Lb.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 28 | 55 | 99 | 117 | 133 | Initial | Final | |
| | | | | | *K 1 (steers)* | | | | | | |
| 26 | Control | 99 | 15.0 | 27.4 | 16.6 | 3.6 | | | 711.9 | 922.0 | 2.0 |
| 24 | Ronnel | 99 | 7.6 | 1.4 | .1 | .96 | | | 769.6 | 987.6 | 2.0 |
| | | | | | *J 1 (steers)* | | | | | | |
| 24 | Control | 117 | 26.6 | 31.6 | 16.3 | | 2.0 | | 588.4 | 850.4 | 2.4 |
| 26 | Ronnel | 117 | 14.4 | 4.8 | .5 | | .9 | | 586.5 | 857.7 | 2.3 |
| | | | | | *BJ1 (heifers)* | | | | | | |
| 24 | Control | 133 | 21.3 | 19.9 | 8.3 | | | 4.3 | 504.8 | 752.3 | 1.8 |
| 25 | Ronnel | 133 | 14.6 | 2.8 | .1 | | | 3.4[b] | 513.6 | 745.8 | 1.9 |

[a] The low count in the control animals near the end of the trials was due to the emergence of grubs from the backs.
[b] The increase in number of grubs from earlier count is believed to result from movement of previously undetected grubs into the back.

Control of Grubs.—The total number of grubs found in each animal at each count was recorded. No evidence of heel fly activity was observed during the course of the trials; all grubs found, therefore, were presumed to have been present in the animals at the time they arrived at the feed lot.

Most of the treated animals showed a marked decrease in the number of grubs present 28 days after the initial counts were made, whereas the untreated animals showed a marked increase in total grub count. Subsequent grub counts showed a decrease in the number of grubs present in the untreated animals. This reduction was caused by the emergence of mature grubs from the animals. Only a few grubs emerged from the treated animals. A comparison of average grub counts at time of treatment and subsequent intervals is presented in table 1.

Weight Studies.—The average daily gain of the treated and control animals is also shown in table 1. In these trials weight gain response from treatment was variable. No significant differences in gain of the treated cattle over the controls were noted. This agrees with Jones & Matsushima (1959) who reported that they obtained no significant differences in weight gains between treated and control animals in studies conducted in Nebraska. The ration used comprised feeds normally fed in Imperial Valley beef rations; it consisted of 70% concentrate feeds and 30% roughage.

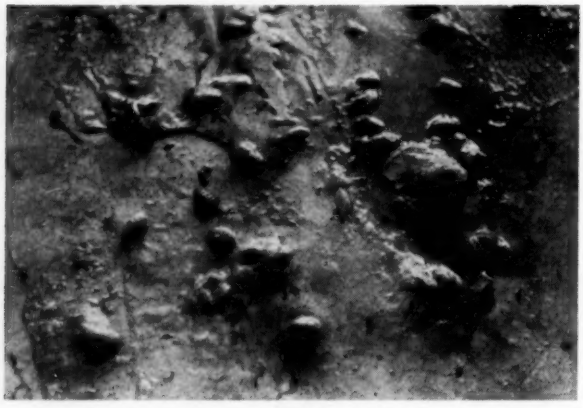

Fig. 1.—Portion of steer hide heavily infested with cattle grubs, *Hypoderma lineatum* (De Vill.). (Typical untreated control animal.)

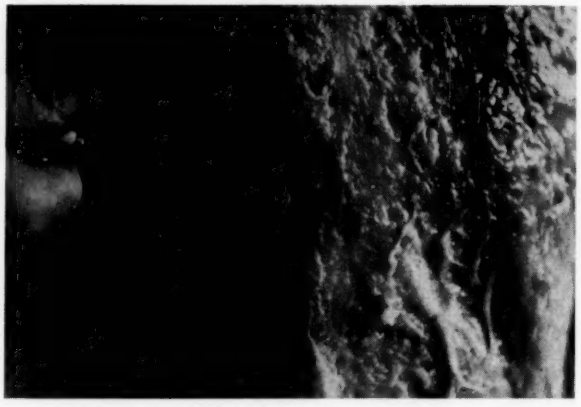

Fig. 2.—Portion of hide from ronnel-treated steer. Note single encysted grub. (Typical of treated animals.)

Discussion and Conclusions.—Ronnel administered orally was shown to be effective against both species of cattle grubs in feed-lot cattle even in animals treated long after the grubs encysted. Timing of treatment was shown to be of primary importance. Visual observations indicate that best results would be obtained in those animals treated before the grubs reached the back. No adverse reactions were noted following treatment.

Hide damage was greatly reduced on the treated animals as compared with the controls (figs. 1 and 2). No carcass trim was needed on the treated animals, but considerable trim was necessary on the untreated animals because of extensive grub injury. There was some indication that treatment with ronnel resulted in slightly better control of grubs in steers than in heifers.

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# Influence of Grain Moisture and Storage Temperature on the Effectiveness of Malathion as a Grain Protectant[1]

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ABSTRACT

The influence of moisture on malathion applied at 10 p.p.m. to wheat with a graded moisture range of 10%, 12%, 14%, 16%, 18%, and 20% and stored at 60° F. was recognized by reduced mortalities of *Sitophilus granarius* (L.), *Sitophilus oryza* (L.), and *Tribolium confusum* Duv. with increases in moisture content. Results indicated that a moisture content of 12% is about the maximum safe level and 14% appeared to be the critical level of moisture in wheat when considered in regard to the persistence of biologically effective malathion deposits. Effectiveness of malathion applied at 10 p.p.m. to wheat of 10% moisture content and stored in temperature-controlled cabinets at graded ranges of 60°, 70°, 80°, 90°, 100°, 110°, and 120° F. decreased with increases in temperature, but maximum safe temperature ranges which may determine the persistence of malathion at effective levels on grain in storage were not clearly defined. Results from experiments concerned with the interrelation of moisture content, storage temperature, and dosages applied indicated the desirability of adjusting dosages of malathion to compensate for adverse effects of moisture content and storage temperatures that may be found in actual storage of grains.

Grain protectants are limited primarily to insecticides with low mammalian toxicity that are effective against storage pests without leaving harmful chemical residues or altering the flavor, odor, or color of the treated grain or finished cereal products. Malathion has properties that permit it to be used for this purpose, but variables limiting the value of malathion as well as those contributing to increased effectiveness must be considered in order to make the most efficient use of this insecticide for the protection of stored grain.

Data presented by Lindgren *et al.* (1954) in the first published report on the use of malathion for the control of insects infesting stored grain showed malathion to be effective for 2 months against the granary weevil (*Sitophilus granarius* (L.)), the rice weevil (*Sitophilus oryza* (L.)), and the lesser grain borer (*Rhyzopertha dominica* (F.)) when applied at 2 p.p.m. to wheat containing 10% moisture. It remained effective for 6 to 7 months when applied at 8 and 16 p.p.m. In other experiments conducted by Lindgren (Gunther *et al.* 1958), malathion was ineffective against these species within 2 months after treatment with dosages of less than 8 p.p.m. on wheat of 12% moisture content.

The effectiveness of malathion treatments was much reduced after storage for 1 or 2 months when used with wheat of 14.5% moisture as compared with that of 9% in experiments with grain protectants for the control of the khapra beetle, *Trogoderma granarium* Everts (Lindgren *et al.* 1955). Watters (1959) also found wheat moisture to be an important factor influencing the effectiveness of malathion against storage pests. In his experiments, malathion applied at 2 p.p.m. to wheat of 13.5% moisture content caused 99% mortality of the rusty grain beetle, *Laemophloeus ferrugineus* (Steph.), at 8 months after treatment; a dosage of 16 p.p.m. was required for comparable control in wheat of 18% moisture at 5 months after treatment. Wilbur (1952) and Schroeder (1955), working with a combination of piperonyl butoxide and pyrethrins, found that as the moisture content of grain was increased the survival and reproduction of *Sitophilus oryza* increased.

Parkin (1958) directed attention to the need for more detailed assessment of the stability of malathion to heat, moisture, and alkalinity in his provisional assessment of malathion for stored-products insect control. However, any factor or combination of variables contributing to storage losses by favoring development of insect infestations might limit the effectiveness of an insecticide applied to grain for protection against storage pests. Although consideration must be given to the moisture content and storage temperature, the kind and condition of grain, including the status of infestations at the time of harvest, should not be overlooked. Susceptibility of species to the insecticide, formulations, and coverage certainly should be added to the list of variables which need

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more detailed assessment. Detailed investigation of factors influencing the effectiveness of insecticides as grain protectants obviously can best be done in the laboratory.

It is the purpose of this paper to present results from experiments concerned with the influence of moisture content, storage temperature, and the interrelation of moisture, temperature and dosage on the effectiveness of malathion in protecting wheat against three major pests of stored grain. This is the first in a series of reports on factors which may influence the effectiveness of malathion as a grain protectant. Subsequent reports on studies in progress at this time will consider the kind and quality of grains, formulations, coverage, and susceptibility of various species of storage pests.

MATERIALS AND METHODS.—Studies on the influence of moisture and temperature on the effectiveness of malathion as a grain protectant were made simultaneously; later the interrelation of moisture, temperature, and dosages of malathion was considered. Materials and methods were the same for each phase of the problem with the exception of those given under specific headings which follow the description of general procedures.

Careful adjustment of moisture and conditioning of wheat was required to obtain samples with the desired percentages of moisture. The moisture content of wheat was determined with an *"EH" Universal Moisture Tester* made by the Moore-Milford Corporation, Evanston, Illinois. Initial moisture tests indicated the need for addition of water or drying of wheat. Water was added as needed to increase moisture. To reduce moisture, wheat was dried in narrow wire-screen containers placed in a forced-air oven maintained at 125° to 130° F. Following each moisture adjustment, wheat was conditioned in sealed containers for 2 weeks at 50° F. Then moisture tests were made. Addition of water or drying, alternated with the conditioning period before moisture tests, was continued until the desired percentage of moisture was obtained, within limits of $\pm 0.2\%$, in each lot of wheat tested.

Technical malathion in acetone was used in all spray preparations. Sprays were prepared from freshly made stock solutions with a concentration of 10 mgs. of malathion per milliliter immediately before application to the grain. Since 1 mg. of malathion applied to 1 kg. of wheat would give a dosage of 1 p.p.m., only simple calculations were necessary to use the stock solution for preparation of sprays that would contain the amount of malathion needed in the total spray to give the desired dosage when applied to a known amount of wheat.

A filling hopper ordinarily used in grain testing for weight per bushel measurements held wheat to be sprayed. Spray was applied into the grain stream with a nasal atomizer as the wheat flowed from the filling hopper into a grain pan. The atomizer was equipped with constant air pressure. The sliding plate valve at the bottom of the filling hopper was opened only enough to allow the flow of grain to coincide with the application of spray. A continuous flow was maintained by agitating the grain slowly over the valve opening at the bottom of the filling hopper with a laboratory stirrer.

Sprayed wheat was left overnight in open polyethylene bags at room temperature before blending for 15 minutes in a *Twin Shell Blender* (Manufactured by the Patterson-Kelley Co., Inc., East Stroudsburg, Pa.) of 8-quart capacity. After blending, each lot of wheat was divided into eight samples of equal size to be used for toxicity tests at intervals of 1 day, 2 weeks, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months after spraying. These samples, with the exception of those used for tests 1 day after spraying, were kept in airtight, doubled, polyethylene bags inside temperature-controlled cabinets. Temperatures were maintained within $\pm 1°$ F. of the levels desired.

One lot of sprayed wheat represented one treatment replicate; treatments were replicated three times in all experiments. Unsprayed wheat was used in all tests as control standards for comparison.

The effectiveness of malathion was tested at the eight intervals designated for aging of deposits by confining 100 adults each of the granary weevil, the rice weevil, and the confused flour beetle, *Tribolium confusum* Duv., in approximately 50 grams of wheat from each treatment replicate. Samples for toxicity tests were held in cloth-covered wide-mouth glass jars. After an exposure of 14 days during which the test samples were kept in a testing room with a temperature of $80° \pm 1°$F. and a relative humidity of $60\% \pm 5\%$, the wheat was screened and insect mortalities recorded.

Granary and rice weevils were reared in wheat with an initial moisture content of 14.5%. Culture media for the confused flour beetle was ground to pass through a 20-mesh screen and consisted of a mixture by weight of whole wheat flour—30 parts, corn meal—10 parts, wheat germ—1 part, dried skim milk—1 part, and brewers yeast—½ part. Parent insects were left in cultures for an oviposition period of 7 days. Insects were used for toxicity tests 14 days after the first adults appeared in cultures, giving test insects with a mean adult age of 7 days and a range in age of 1 to 14 days. The temperature in the culture room was maintained at $80° \pm 2°$ F. and the relative humidity at $60\% \pm 5\%$.

General procedures followed in all experiments were described in the preceding paragraphs. Materials and methods employed only in studies concerning particular phases of the problem are given below.

*Influence of Grain Moisture.*—White Federation wheat containing a high percentage of damaged kernels, dust, and insect fragments was used; the wheat had been heavily infested with the lesser grain borer. Moisture adjustments were made as necessary to obtain wheat with the desired graded range of 10%, 12%, 14%, 16%, 18%, and 20% moisture content. Spray was applied at the rate of 10 ml. of total spray on 1200 gms. of wheat to give a dosage of 10 p.p.m. malathion. Samples were stored at 60° F. Tests on the effectiveness of malathion were made at regular intervals as mentioned previously.

*Influence of Storage Temperature.*—Moisture was adjusted to obtain a uniform moisture content of 10% in all test samples of wheat. Samples were stored in temperature-controlled cabinets at graded ranges of 60°, 70°, 80°, 90°, 100°, 110°, and 120° F. The kind of wheat used, dosage of malathion, and application of spray were the same in this as in the preceding experiment.

*Interrelation of Moisture, Temperature, and Dosages of Malathion.*—Each of three dosages of malathion (10, 20,

and 40 p.p.m.) was applied to wheat of three moisture levels (10%, 12%, and 14% moisture content) and the treated wheat was stored at three temperatures (60°, 90°, and 120° F.). Spray was applied to give the desired dosage of malathion in 15 ml. of total spray on 2700 gms. of wheat. Ramona wheat in excellent condition was used; the wheat had few broken kernels and very little dust, and had not been infested with insects.

RESULTS.—The mortality of adult insects was the only criterion used to measure the effectiveness of malathion as a grain protectant under various storage conditions. Although the mortality of insects in unsprayed control samples of wheat was generally low, results were corrected by the use of Abbott's formula (1925). The corrected percentage mortalities are presented graphically for ease in making comparisons. Only a summary of the data essential to the understanding and interpretation of results are given. Those data not presented in the graphs are mentioned, however.

*Influence of Grain Moisture.*—Results from experiments concerned with the influence of moisture on the effectiveness of malathion are summarized in figure 1. Graphs presented in figure 1 depicting results obtained with each species of test insects are as follows: (A-1) *Sitophilus granarius*, (A-2) *Sitophilus oryza*, and (A-3) *Tribolium confusum*. Each graph projects the corrected percentages of mortality (numbers on top of bars) caused by 10 p.p.m. malathion in wheat containing varying amounts of moisture (moisture content—%) after storage at 60° F. for the periods shown (age of deposit).

Although data are presented for only six intervals following application of malathion spray, assessment of the effectiveness of malathion was made at each of the eight intervals mentioned previously. Malathion caused 100% mortality of *Sitophilus granarius* and *S. oryza* in all samples tested 1 day and 2 weeks after application of spray as one would suspect from results given for 1-month-old deposits (fig. 1, A-1 and A-2). Excessive mortality of control insects due to overpopulated cultures occurred in tests with *Tribolium confusum* 3 months and 9 months after application of spray. Results obtained at these test intervals were considered invalid and are not presented. Mortalities recorded for *T. confusum* adults in control samples in all other tests were very low.

*Influence of Storage Temperature.*—Results from experiments on the influence of storage temperature on the effectiveness of malathion are summarized in figure 1. Graphs presented in figure 1 illustrate results obtained with each species of test insects as follows: (B-1) *Sitophilus granarius*, (B-2) *Sitophilus oryza*, and (B-3) *Tribolium confusum*. Each graph projects the corrected percentages of mortality (numbers on top of bars) caused by 10 p.p.m. malathion in wheat of 10% moisture content after storage at various temperatures (storage temperature—°F.) for the periods shown (age of deposit).

Although not shown in the graphs (fig. 1, A-1 and A-2), malathion caused 100% mortality of *Sitophilus granarius* and *S. oryza* in all samples tested 1 day after application of spray. Results from tests made with *Tribolium confusum* 3 months and 9 months after application of spray are not presented for the same reason results were not given for tests at these intervals in the preceding study on the influence of grain moisture on the effectiveness of malathion.

*Interrelation of Moisture Content, Storage Temperature, and Dosages of Malathion.*—Part of the results obtained from tests with three species of insects in experiments concerning the interrelation of moisture content, storage temperature, and dosages of malathion are given in figures 2 to 4. The influence of moisture, temperature, dosage of malathion, or a combination of conditions, on the effectiveness of malathion in protecting wheat against insects is apparent from horizontal and vertical examination of the data presented for each species tested. Comparisons between species may be made by referring from one figure to another.

Although tests were made at each of the eight intervals mentioned in the general description of materials and methods, only data from selected intervals were used to prepare the graphic summary of results. For this reason, the ages of malathion deposits shown in figures 2 to 4 varies between temperatures. However, sufficient direct comparisons can be made to indicate the relative influence of storage temperatures on the effectiveness of various dosages of malathion on wheat of varying moisture contents. A brief descriptive summary of results not presented in the graphs should make comparisons more complete.

Results presented in figures 2 and 3 for malathion deposits aged for 12 months at 60° F. are sufficient to indicate the effectiveness of malathion against *Sitophilus granarius* and *S. oryza* on wheat stored for shorter periods. Against *Tribolium confusum* there was a graded decrease in the effectiveness of malathion on wheat stored at 60° F. in tests made at intervals between those shown in figure 4.

In wheat stored at 90° F. malathion was very effective against *Sitophilus granarius* and *S. oryza* in tests made 1 day, 2 weeks, and 1 month following application of spray as one would expect from results presented in figures 2 and 3 for 2-month-old deposits. Different results were obtained with *Tribolium confusum*. Mortalities recorded for this species in wheat treated with 10 p.p.m. malathion decreased gradually from 100% in tests made 1 day after application of spray to the mortalities recorded in figure 4 for deposits aged 2 months. Tests made 1 month after treatment resulted in slightly less than 100% mortality of *T. confusum* on wheat sprayed with 20 p.p.m. malathion and stored at 90° F. Where the insecticide was not completely effective against *T. confusum* prior to the 2-month-test-interval, a graded decrease in mortalities occurred which corresponded to the graded increase in the moisture content of test samples.

All treatments were ineffective at the end of 6 months on wheat stored at 120° F. Data presented in figures 2 to 4 indicate the rapid loss in effectiveness of malathion on grain stored at this temperature.

DISCUSSION.—A comprehensive review of principles governing storage of cereal grains may be found in the monograph edited by Anderson & Alcock (1954). In this book recognized authorities elucidate and illustrate the importance of moisture, temperature, and other factors concerned in the storage of cereal grains and their products. Thus, there is no reason to expand the present dis-

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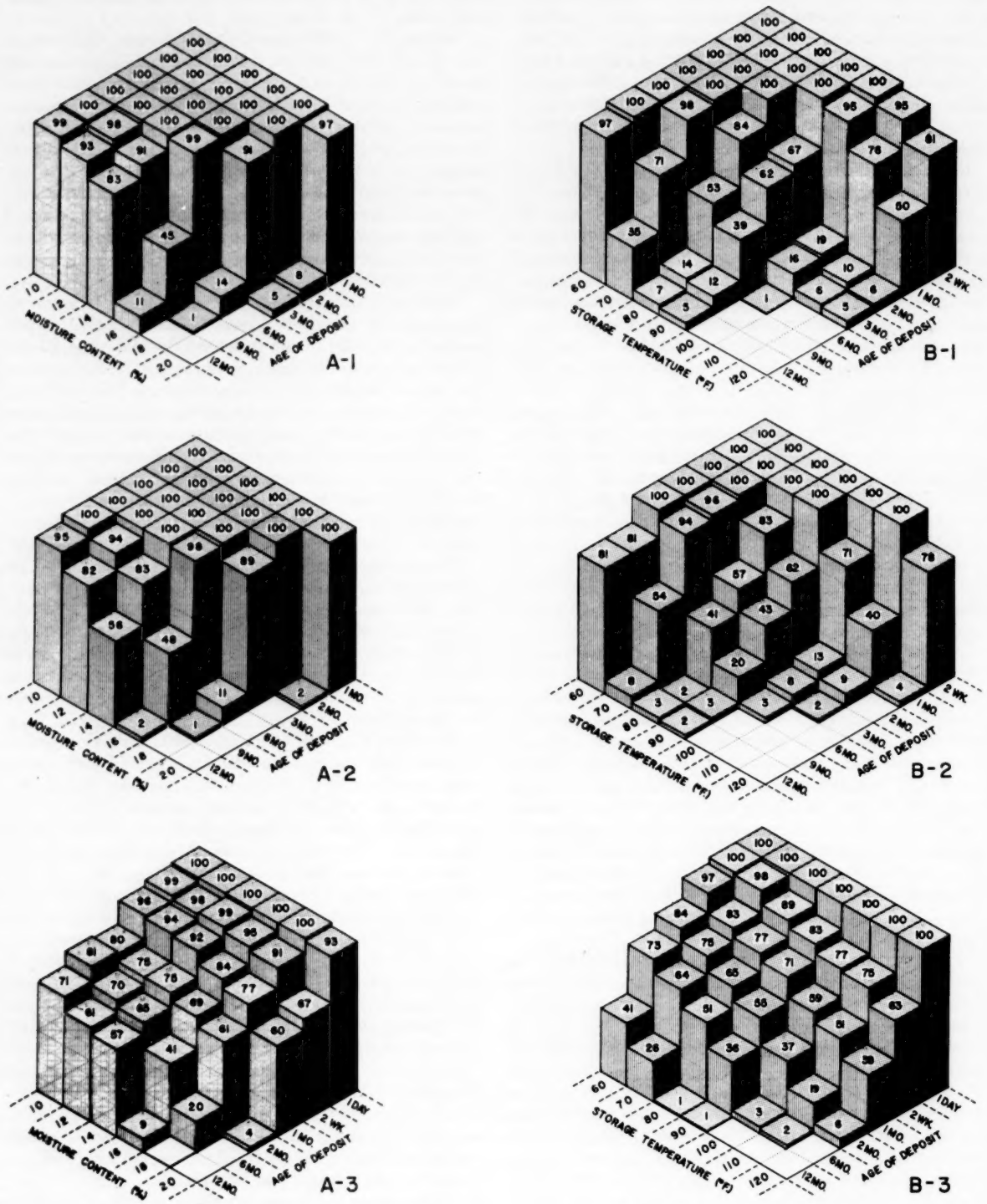


FIG. 1.—Influence of moisture content (A-1 to A-3) and storage temperature (B-1 to B-3) on the effectiveness of malathion applied at 10 p.p.m. to White Federation wheat of poor quality as indicated by corrected percentage mortalities (numbers on top of bars) of three species of insects after an exposure of 2 weeks in treated wheat at various intervals (age of deposit) after application of spray: (A-1) *Sitophilus granarius* (L.), (A-2) *Sitophilus oryza* (L.), and (A-3) *Tribolium confusum* Duv.; (B-1) *Sitophilus granarius* (L.), (B-2) *Sitophilus oryza* (L.), and (B-3) *Tribolium confusum* Duv.

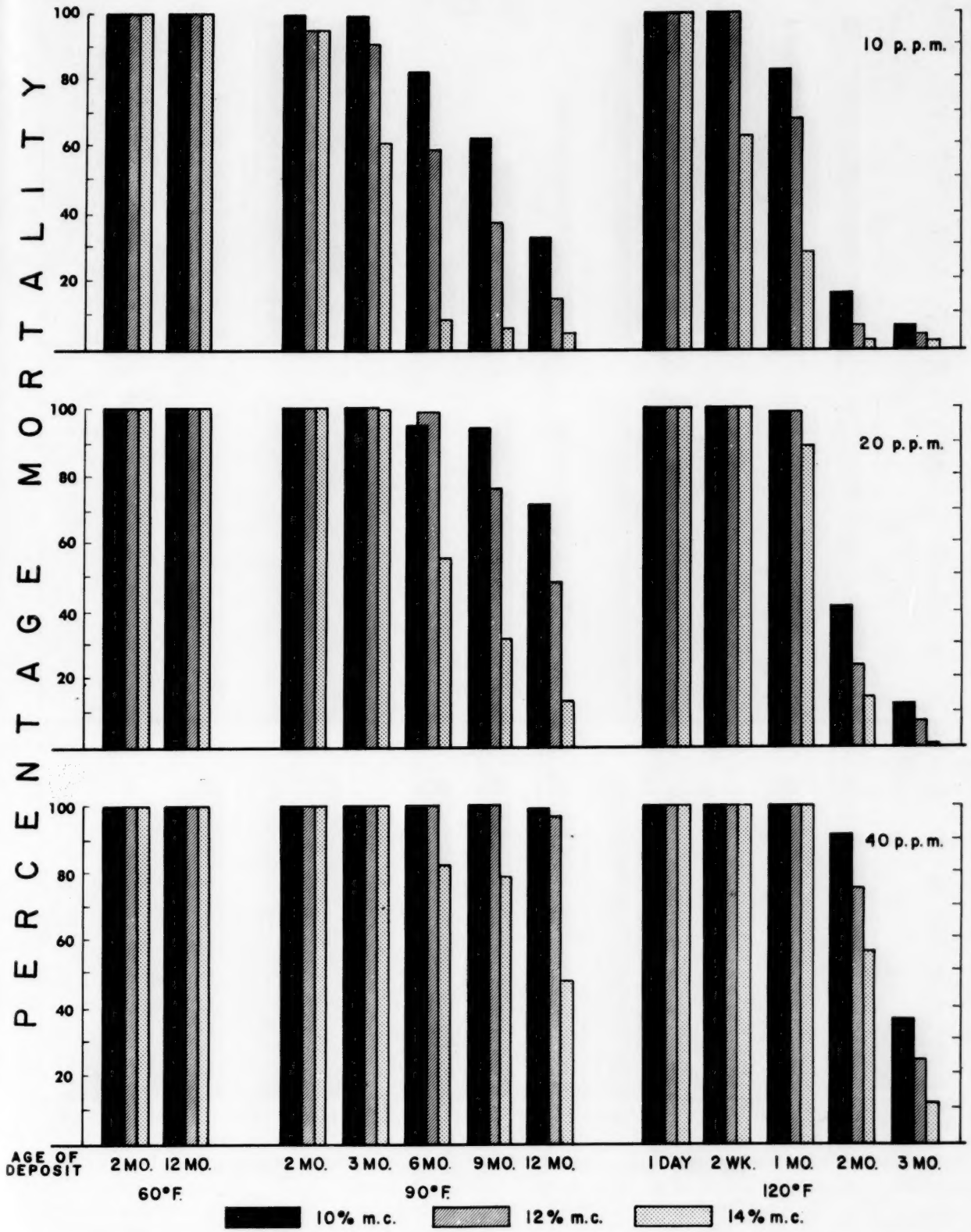


FIG. 2.—Interrelation of moisture content (m. c.), storage temperature (° F.), and dosage (p.p.m.) on the effectiveness of malathion in protecting Ramona wheat of good quality against *Sitophilus granarius* (L.) as indicated by the corrected percentage mortality of adult insects exposed to treated wheat for 2 weeks at various intervals (age of deposit) after application of malathion spray.

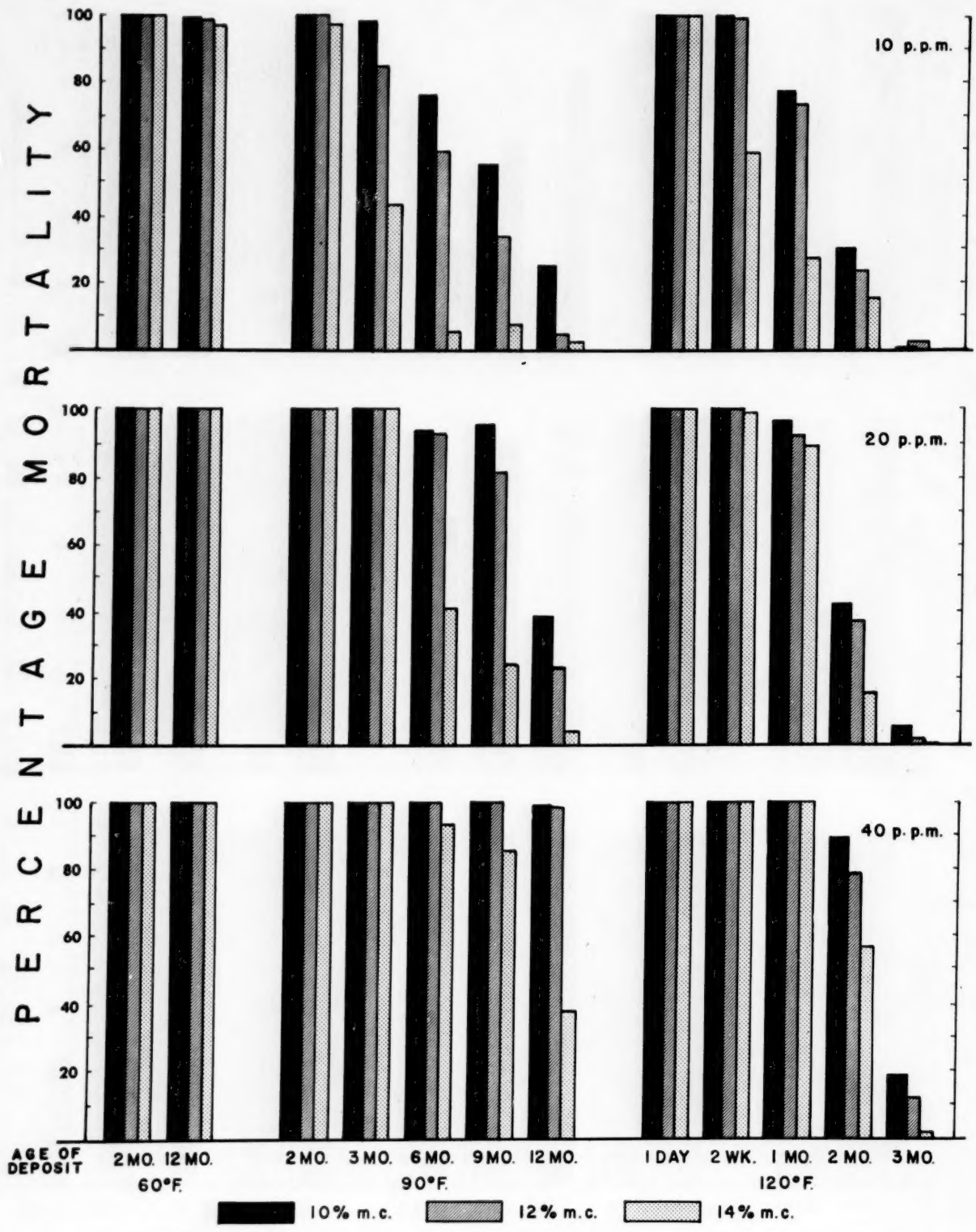


FIG. 3.—Interrelation of moisture content (m. c.), storage temperature (° F.), and dosage (p.p.m.) on the effectiveness of malathion in protecting Ramona wheat of good quality against *Sitophilus oryza* (L.) as indicated by the corrected percentage mortality of adult insects exposed to treated wheat for 2 weeks at various intervals (age of deposit) after application of malathion spray.

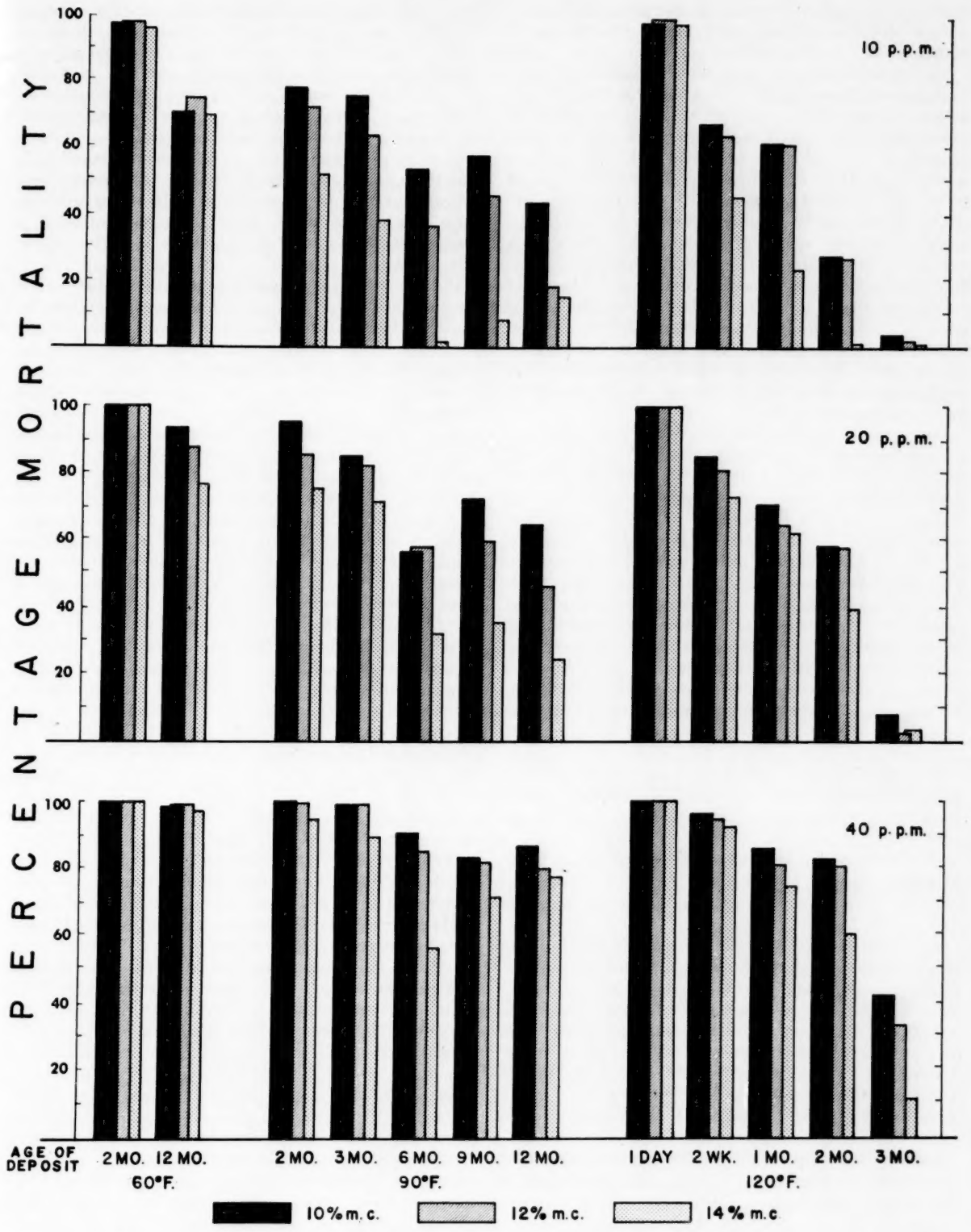


FIG. 4.—Interrelation of moisture content (m. c.), storage temperature (° F.), and dosage (p.p.m.) on the effectiveness of malathion in protecting Ramona wheat of good quality against *Tribolium confusum* Duv. as indicated by the corrected percentage mortality of adult insects exposed to treated wheat for 2 weeks at various intervals (age of deposit) after application of malathion spray.

cussion of results much beyond the influence of moisture content and storage temperature on the effectiveness of malathion as a grain protectant.

The influence of moisture on the effectiveness of malathion against storage pests is recognized by reduced mortalities of insects with increases in the moisture content of wheat (fig. 1, A-1 to A-3). A moisture content of 12% appears to be the maximum safe level and 14% moisture appears to be the critical level when considered in regard to the persistence of biologically effective malathion deposits. Although grain usually would not be stored with moistures as high as the extremes in these tests, such moisture levels might be found at the time of harvest. Should this happen, the moisture content probably would be reduced either in or immediately before placing the grain in storage. Malathion, according to the data presented, could be expected to give protection against storage pests over a longer period of time if applied after the moisture content was reduced to levels considered safe for storage of the grain.

Decreases in the residual effectiveness of malathion with increases in storage temperature are apparent from data presented in figure 1 (B-1 to B-3). Graded decreases in effectiveness comparable to graded increases in temperature are seen in the summary of results from tests with *Tribolium confusum* (fig. 1, B-3). More abrupt reductions in mortalities of insects were obtained in tests with *Sitophilus granarius* (fig. 1, B-1) and *S. oryza* (fig. 1, B-2), but maximum safe temperature ranges which may determine the persistence of malathion at effective levels on grain in storage are not clearly defined. The results indicate, however, that the effectiveness of malathion as a protectant against insect infestations in stored grain may be dependent on the temperature of grain at the time malathion is applied and its temperature in storage afterwards.

The preceding discussion concerned results from experiments in which all variables were constant except those under consideration, but the influence of moisture content and storage temperature on the effectiveness of malathion could not be isolated so easily in actual storage of grains. For this reason, the interrelation of moisture content, storage temperature, and dosages of malathion was explored.

Reference is made to data presented in figures 2 to 4 when considering the interrelation of moisture content, storage temperature, and dosage on the effectiveness of malathion. The results indicate that malathion applied at the rate of 10 p.p.m. would remain effective against insect infestations for at least 12 months when storage temperatures are no higher than 60° F. and the moisture content of wheat does not exceed 14%. Storage temperatures as high as 120° F. limited the effective life of malathion so severely that the moisture content of the grain stored at such temperatures would be of little concern although the data presented indicate even more rapid impairment in effectiveness of the insecticide at this temperature with increases in moisture content.

The interrelation of moisture and temperature on the persistence of malathion deposits in grain is readily apparent in graphs depicting results from tests with malathion-treated wheat stored at 90° F. The combined influence of moisture and temperature is especially obvious in those data presented from tests with *Sitophilus granarius* (fig. 2) and *S. oryza* (fig. 3). Lowered mortalities recorded from tests with insects in grain that had been treated with malathion and stored at 90° F. were not considered to be caused entirely by either moisture or temperature. If moisture had been the sole contributor to decreased effectiveness, moisture effects should have been more pronounced in tests with grain sprayed with malathion and stored at 60° F. If temperature alone were the causative factor, wheat sprayed with 40 p.p.m. malathion and held at 90° F. should have had comparably lower mortalities of test insects in grain of all three moisture levels.

Data presented in figures 2 to 4 indicate that by increasing the dosages of malathion compensation may be made for the adverse effects of moisture and temperature, yet it is evident that limits may be reached at which increased dosages would not materially prolong the effectiveness of malathion as a grain protectant. Watters (1959) found this fact to be true in his study on the effects of moisture content on the toxicity of malathion. Such limits apparently exist, however, beyond the range of conditions suitable for safe storage of grains.

From a practical viewpoint, application of malathion at dosages designed to compensate for adverse effects of moisture and temperature is worthy of consideration. Dosages of malathion approaching the permissible residue tolerance of 8 p.p.m. may provide ample protection of grain under certain conditions, while, according to results from tests in this study, considerably more malathion must be used to provide equivalent protection against insects under more adverse conditions. Further emphasis is given to the desirability of applying dosages of malathion beyond the level of permissible residue tolerances by an examination of results reported by Womack & LaHue (1959) from tests with malathion for the protection of shelled corn. Compensatory adjustment in dosages of malathion probably should not be recommended as a common practice, however, until sufficient data are provided from chemical residue analyses to permit correlation of dosages applied with residues remaining in grain of various moisture contents stored at temperatures most likely to be found in actual storage of grains.

The three species of insects used in this study were selected intentionally because known differences in their habits, distribution, and importance as pests of stored grain should be of value in the interpretation of results in terms of actual storage of grain in a particular location. Furthermore, differences were expected to be found between species in the performance of malathion as measured by the mortality of adult insects exposed to treated wheat. This expectation was based on results presented by Vincent & Lindgren (1957) in which 11 times more malathion was required for 95% kill of *Tribolium confusum* than for *Sitophilus granarius* with topical applications of insecticide. Data presented in figures 1 to 4 indicate that *T. confusum* is somewhat less susceptible than *S. granarius* and *S. oryza* to malathion-treated wheat. Results from tests with *S. granarius* and *S. oryza* were similar for both species.

Wheat of poor quality containing a high percentage of damaged kernels, dust, and insect fragments was used in experiments considering the influence of moisture and

temperature on the effectiveness of malathion. Later, when the interrelation of moisture, temperature, and dosages of malathion was explored in a series of tests, wheat in excellent condition and of another variety was obtained. Differences between wheats used does not alter the validity of results given in figures 1 to 4 since the problem was divided into three distinct phases of study and the condition of wheat was constant in each. It does, however, provide an opportunity to make qualitative observations on the influence of condition of grain on the effectiveness of malathion. Results from tests with wheat of 10%, 12%, and 14% moisture content sprayed with 10 p.p.m. malathion and stored at 60° F. may be compared for each species of insects by referring from figure 1 (A-1 to A-3) to the appropriate graphs in figures 2 to 4. Differences are most obvious in tests with wheat of 14% moisture content. Comparisons may be made between temperature effects on malathion in wheat of poor condition and good condition by referring to the data presented in figure 1 (B-1 to B-3) and comparing results given with those presented in figures 2 to 4 for wheat of 10% moisture content sprayed with 10 p.p.m. malathion and stored at 60°, 90°, and 120° F. The differences observed may be attributed to the quality of wheat and emphasizes the necessity of describing the condition of grain used in tests of this kind.

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# Influence of Timing and Insect Biology on the Effectiveness of Insecticides Applied for Control of European Pine Shoot Moth, *Rhyacionia buoliana*[1]

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## Abstract

Spring and summer field insecticide applications were made to compare the effectiveness of selected chlorinated hydrocarbon and organic phosphate insecticides in control of the European pine shoot moth. Control obtained with spring applications of dimethoate, DDT, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) confirmed earlier success with these materials. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl phosphorodithioate); methoxychlor; and Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) also showed promise. Evidence that resumption of spring feeding and larval migration may be protracted by weather in a given year suggests that timing of the spring insecticide application should coincide with initial pitch blister ("tent") formation. Results of Sevin® (1-naphthyl-N-methylcarbamate), DDT, and dimethoate applications made seven times between the period of 60% adult emergence and the date when 90% of the eggs had hatched (summer) give evidence that treatment should be deferred until at least 50% of the eggs have hatched. Evidence showing that larvae always feed first in the needles and then in the buds of the tree points up the desirability of having a maximum amount of insecticidal residue present at this time.


Currently, Michigan is growing some 160 million conifers for the Christmas tree market (James 1959). Scotch pine (*Pinus sylvestris* L.) comprises 58% of this total and red pine (*Pinus resinosa* Ait.) 19%. Pine species represented in the remaining 23% are jack pine (*P. banksiana* Lamb.); Austrian pine (*P. nigra* Arn.); and white pine (*P. strobus* L.). All pine species are subject to attack by the European pine shoot moth (*Rhyacionia buoliana* (Schiff.)), with red and Scotch sustaining most severe injury. The shoot moth winters in or adjacent to terminal and lateral buds as a partially grown larva and resumes feeding about April 15 in Southern Michigan. Pupation takes place about

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May 15, and egg hatching begins about the last week of June.

Miller & Neiswander (1955) summarized the literature pertaining to biology and control of the European pine shoot moth and reported on investigations carried out in Ohio between 1950 and 1953. They concluded that DDT sprays applied in mid-April and late June or early July would give excellent control. The unpredictable results achieved by many growers in Michigan since then, coupled with the economics of the tree farming operation, have sustained interest in new insecticides and in application with low-gallonage equipment.

Guyer *et al.* (1957) and Morofsky *et al.* (1958) confirmed the superiority of DDT in both spring and summer experiments. Haynes *et al.* (1958) experimented with organic phosphate insecticides applied as: (1) foliar sprays; (2) soil treatments; and (3) insecticide saturated bands applied to the trunk in the fall of 1956 and in the spring of 1957. In these studies, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) gave the most rapid kill, while phorate (Thimet) applied in the fall to the soil at the base of the tree gave the greatest population reduction. In the case of phorate, it was concluded that mortality was owing to systemic action of the insecticide.

The possible usefulness of Phosdrin as a substitute for fumigation of seedlings being lifted for fall shipment was suggested in work done by Butcher & Haynes (1958). In dorsal dermal contact studies, Haynes (1959) found that organic phosphates would kill overwintering larvae at concentrations where chlorinated hydrocarbons had no appreciable effect. Butcher & Haynes (1959) found that DDT, dimethoate, Sevin® (1-naphthyl-N-methylcarbamate), and Phosdrin drastically reduced populations of European pine shoot moth when applied at the time larvae resumed spring feeding. Summer applications of these and other materials gave erratic results. However, success with DDT and dimethoate in delayed applications encouraged further investigations on the proper timing of promising materials. The results of these investigations are reported in this paper.

EXPERIMENTAL METHOD.[2]—*Description of Host Trees and Equipment.*—All trees used in these experiments were plantation grown red pine. The trees averaged 5 to 7 feet in height, with the exception of one stand at Mason, Michigan, where the average height was slightly less than 5 feet. All insecticides were applied with a 2-gallon Lofgren hydraulic sprayer equipped with a fan type nozzle, and operated at 15 to 25 p.s.i. This kind of equipment is not practical for use on large numbers of trees or even on large trees. It served the dual purpose in these studies of (1) permitting coverage of individual trees with a minimum of drift; and (2) minimizing the influence of application equipment in the study. It may be assumed that control by any of the materials would be even better if they were applied with high volume, high pressure equipment.

*Design of Experiment.*—Single tree plots were used throughout with each treatment replicated as follows:

| | *No. of Replications* | |
|---|---|---|
| *Plot Location* | *Spring* | *Summer* |
| Mason | 10 | — |
| E. Lansing | 9 | 8 |
| Mesick | 10 | 10 |

In all experiments, each treatment and check occurred once per replication in a randomized block design.

*Method of Insecticide Application.* The foliage was sprayed until visibly wet, but spraying was stopped before run-off occurred. The technique permitted good coverage of the bud areas, and ten 5- to 7-foot trees could be covered with 1 to 1.5 gallons of spray liquid.

*Population Sampling and Statistical Analysis.* Population counts on the spring plots were obtained from early June field dissection of all shoots showing signs of feeding. At this time pupation was underway but no adult emergence had taken place. These counts indicated the actual number of live shoot moth larvae or pupae on each tree at the time of sampling.

Estimates of live larvae surviving in the summer treatments were arrived at by making field counts in October of infested buds (number infested out of the top 50 buds on each tree). In addition, five 2-inch shoot tips were cut at random from the top 50. These were packaged, labeled, and taken into the laboratory for dissection under magnification. In this way an average number of live shoot moth larvae per 2-inch shoot tip was determined. The product of number of infested buds in sample and average number of live larvae per infested 2-inch shoot tip yielded a population estimate for the upper 50 shoots of each plot. This permitted comparisons between treatments. These data were subjected to analyses of variance, and maximum significant differences were calculated using the table presented by Duncan (1955).

*Timing of Insecticide Applications.* Spring screening of candidate materials was carried out at three locations in Michigan. In each place, applications were made at the time initial "tent" formation became evident.

Summer timing studies were carried out at two locations in Michigan. Candidate materials were applied at weekly intervals for a 3-week period at East Lansing and over a 4-week period at Mesick, 110 miles to the north. Observations on development and behavior were made concurrently in both areas.

A 1959 comparison of pupation, adult emergence, and egg hatch for the two locations revealed that on the average these phenomena occurred 7 days later at Mesick than at East Lansing. As a result, a phenological integration of these data allows a chronological comparison of spray applications. Thus, even though the time interval between applications is not uniform, a total of seven separate replicated comparisons is possible. The first replicated applications were made at the time 60% of the adults had emerged from pupal cases, while the seventh coincided with the date when 90% of the eggs had hatched.

DESCRIPTION OF INSECT LIFE HISTORY AND BEHAVIOR.—The results of these studies can best be interpreted in the light of development and behavior of the shoot moth. Figure 1 is a pictograph, which shows the initia-

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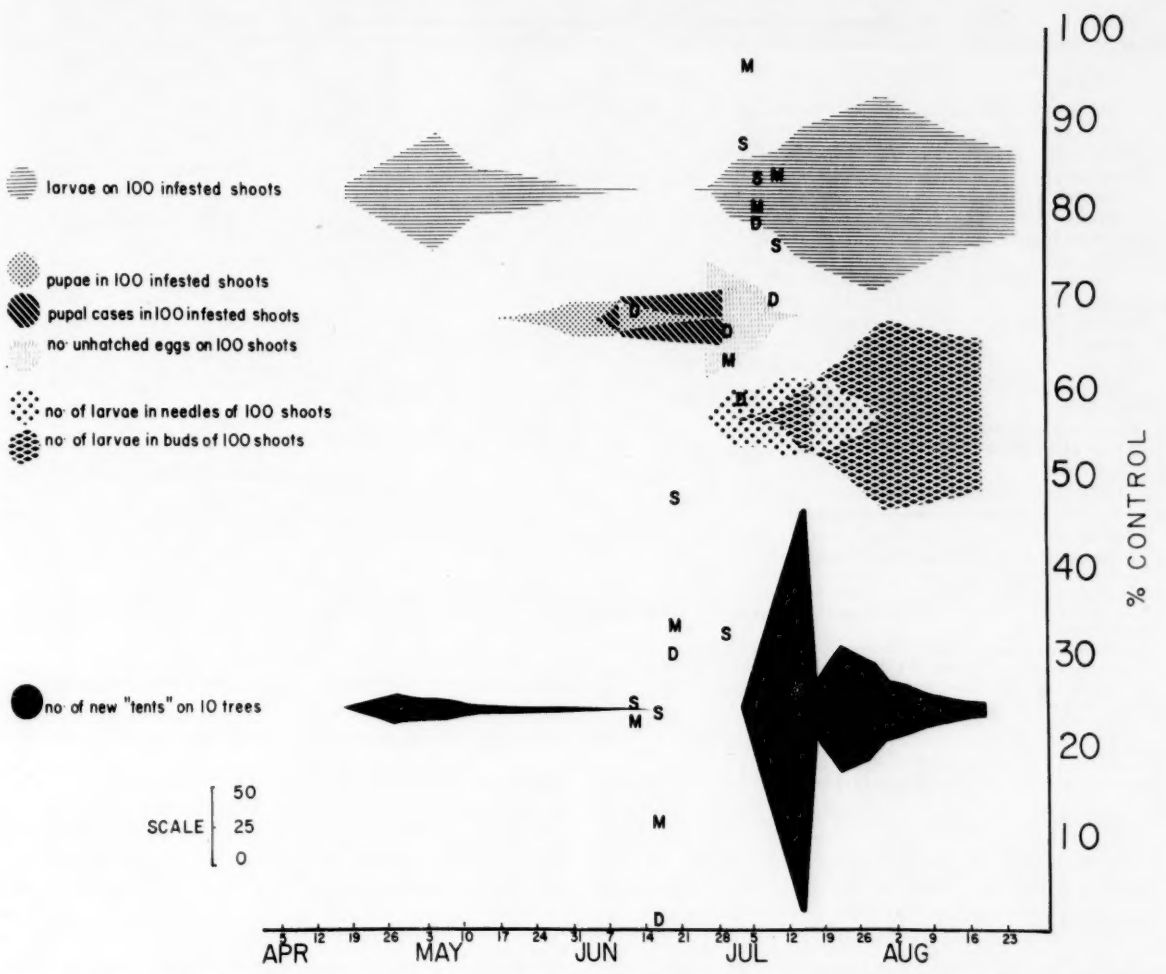


FIG. 1.—Pictograph showing 1959 European pine shoot moth life cycle and behavior phenomena at Mason, Michigan in relation to calendar dates. All observations were made at 3- or 4-day intervals. Symbols M (dimethoate); D (DDT) and S (Sevin) are positioned according to per cent shoot moth control obtained (vertical axis) and date applied (horizontal axis).

tion, progress, and termination of some observable life history and behavioral phenomena. For any particular date, it is possible to ascertain the magnitude of any given phenomenon with respect to any other. Reading from top to bottom, the life history phenomena recorded are (a) live larvae in 100 infested shoots;[3] (b) number of pupae in 100 infested shoots; (c) number of empty pupal cases in 100 infested shoots (a reliable measure of progressive adult emergence); and (d) number of unhatched eggs on 100 shoots. Behavioral phenomena that are significant in control are (e) number of larvae in needles of 100 shoots; (f) number of larvae in buds of 100 shoots, and (g) number of new "tents" (see below) on 10 trees.

Records on larvae, pupae, and pupal cases were obtained from shoot dissections while unhatched egg counts were derived from microscopic inspection of shoots (cut and examined in the laboratory). Dissections under magnification in the laboratory were used to detect the presence of larvae in the needles and buds. "Tents" are formed from silken webbing and pitch accumulated during larval feeding. These are conspicuous and can be observed readily in the field. Once a new tent had been counted it was tagged, so that subsequent counts would include only those formed after the previous observation. All observations were made at 3- or 4-day intervals, and the pictograph configurations represent a line drawn through points (counts) made 3 or 4 days apart, with one-half of the total above and one-half below a horizontal base line.

Of the life history and behavioral phenomena depicted in figure 1, probably the most significant, from the standpoint of summer spray timing, is the evidence that needle feeding and bud injury are events that occur in sequence. Any larva ostensibly feeds in the needle and then in the bud area. This entails considerable movement and feeding on the shoot, needle, or bud surface by all shoot moth larvae subsequent to hatching.

RESULTS OF SPRING APPLICATIONS.—Table 1 lists the

[3] Shoots with pitch blisters were considered infested. Early counts necessarily were made of old blisters ("tents") containing over-wintered dormant larvae, 90% of which were dead. When spring feeding resumed, the fresh blisters were easier to find and contained more live larvae than those made the previous year. The apparent increase in population shown by sampling from April 17 through May 3 is caused by the manner in which infested shoots were selected.

**Table 1.—Influence of insecticides on European pine shoot moth populations. Spring, 1959.**

| Insecticide | Formulation | Toxicant (Lb./100 Gal.) | Live Insects per Tree in June 1959 at | | |
|---|---|---|---|---|---|
| | | | Mason | East Lansing | Mesick |
| Dimethoate | 4 lb./gal. E.C. | 1.0 | 0.0* | 1.1* | 3.3* |
| Phosdrin | 2 lb./gal. E.C. | 1.0 | 0.5* | 1.7* | 3.9* |
| Guthion | 2 lb./gal. E.C. | 1.0 | 0.5* | 2.2* | 3.7* |
| Methoxychlor | W.P., 50% | 2.0 | 1.7* | 2.4 | 3.2* |
| Bayer 22408 | W.P., 50% | 2.0 | 1.1* | 0.4* | 3.1* |
| DDT | 3 lb./gal. E.C. | 2.0 | 1.3* | 3.9 | 3.9* |
| Sevin | W.P., 50% | 4.0 | 0.0* | 1.3* | 4.9 |
| Bayer 29493[a] | 2 lb./gal. E.C. | 0.75 | 3.4 | 5.7 | 3.1* |
| Trithion®[b] | 4 lb./gal. Flow. | 1.0 | 1.3* | 3.9 | 9.8 |
| Ethion | 4 lb./gal. E.C. | 1.0 | 2.5* | 4.3 | 7.0 |
| SD 4402[c] | 1.25 lb./gal. E.C. | 0.5 | 2.1* | 5.3 | 8.6 |
| Shell Sol.+DDT[d] | | 2.0 | 0.3* | — | — |
| Delnav®[e] | 4 lb./gal. E.C. | 2.0 | 6.6 | — | 6.1 |
| *Bacillus thuringiensis*[f] | | 400 gm. | 2.8 | 4.3 | — |
| Orthol D+DDT[g] | | 2.0 | 0.0* | — | — |
| Check | | | 6.5 | 6.0 | 9.0 |
| Difference required for significance at the 5% level[h] | | | 3.80 | 3.74 | 4.84 |

* Differs from check at 5% level of significance (Duncan 1955).

[a] *O,O* dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate.

[b] *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.

[c] Shell Chemical Corp. Compound (1, 3, 4, 5, 6, 7, 8-octachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanophthalan).

[d] An insecticide solvent manufactured by Shell Chemical Co. DDT emulsion concentrate was added at the rate of 2 pounds actual DDT in 100 gallons of final solution.

[e] 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate).

[f] Wettable powder containing 70 billion viable spores per gram. Supplied by Merck & Co., Inc.

[g] Orthol D—soluble oil. Supplied by California Spray Chemical Corp. (DDT emulsion concentrate was added at the rate of 2 pounds actual DDT in 100 gallons of final solution).

[h] Computed from the maximum range of means using Duncan's (1955) multiple range table.

insecticides used in screening trials carried out at Mason, East Lansing, and Mesick, Michigan, in the spring of 1959. Of the materials listed in table 1, dimethoate, DDT, Phosdrin, and Sevin gave control in the spring of 1958 (Butcher & Haynes 1959). In the work reported here, dimethoate performed well at 1 lb. per 100 gallons, as opposed to the 2-lb. rate used in 1958. Phosdrin performed well also, but this chemical is expensive and dangerous to use. This finding adds additional evidence to the suggestion (Butcher & Haynes 1958) that under favorable circumstances Phosdrin may be substituted for gas fumigation in shoot moth control when the larvae are on or in the plant at the time of application.

Guthion® (*O, O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl phosphorodithioate) gave good control at all locations as did Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate). Populations in the plots treated with DDT or methoxychlor differed significantly from the check in two out of three locations. This was also true of Sevin but at an extremely high (4-lb.) dosage. *Bacillus thuringiensis* Berliner, tried at Mason and East Lansing, did not reduce populations significantly at either place.

Two oil formulations, Shell Sol. 72 and Orthol D were used in combination with DDT. The superior wetting qualities of oils made them useful in low gallonage equipment. Good control of shoot moth was obtained with both of these mixtures, although considerable bud injury was noted on the Orthol D plots. Both of these materials were applied full strength, with DDT added at the rate of 2 pounds per 100 gallons.

Heavy kill of overwintering European pine shoot moth larvae was noted in the spring of 1959, although mortality at East Lansing and Mason appeared heavier than that observed at Mesick. To some extent, the method of application undoubtedly influenced the counts between areas (see table 1). Although every effort was made to spray all trees until the foliage was visibly wet, the smaller trees in the Mason plots appeared to have been covered more completely, particularly on the terminal and stem areas. This difference in spraying could explain the fact that, of the materials that were used in all three areas, 10 differed significantly from the check at Mason, whereas only five were significantly different at East Lansing and seven at Mesick.

Overwintered shoot moth larvae form new "tents" or pitch masses upon resumption of spring feeding (about April 15 at Mason in 1959). It has been thought that most of these would be formed within a period of 2 or 3 days. Observations made in the spring of 1959 suggest that this phenomenon might on occasion be prolonged considerably (see figure 1 "Number of new 'tents' on 10 trees"). Here fresh "tents" formed as a result of renewed feeding by overwintering larvae were found over a 10-day period. New "tents" formed by migrating larvae were found over a period of almost 2 months.

Results of experimental applications made in the spring of 1958 (Butcher & Haynes 1959) were consistently better for all effective materials than they were in 1959. This could be attributed to the explosive character of spring "tent" formation noted in 1958, as contrasted to the drawn out pattern of the 1959 activity. Since it appears that these differences can be considerable from year to year, delaying spray applications until first "tent" formation is observed appears to result in more successful control than applications made by the calendar.

Results of Summer Applications—Table 2 lists the insecticides used in timing studies carried out at East

**Table 2.—Influence of insecticides and timing of applications in summer European pine shoot moth control.**

| Insecticide | Formulation | Toxicant (Pounds/ 100 Gal.) | Date of Application: 12 June (ADJ)[a] | 17 June | 19 June (ADJ) | 30 June (ADJ) | 2 July | 6 July (ADJ) | 9 July |
|---|---|---|---|---|---|---|---|---|---|
| | | | Average No. Live Larvae in 50 Shoots in September 1959[b] | | | | | | |
| Dimethoate | 4 lb./gal. E.C. | 2 | 14.1 | 7.3 | 14.6 | 7.0* | 0.2* | 3.4* | 1.3* |
| DDT | 3 lb./gal. E.C. | 1 | 5.5* | 11.5 | 15.3 | 6.3* | 3.2 | 3.9* | 2.7* |
| Sevin | 85% sprayable | 2 | 13.8 | 6.3 | 11.3 | 13.2 | 0.9* | 3.0* | 2.2* |
| Malathion | 5 lb./gal. E.C. | 1 | 10.9 | 10.5 | 15.6 | 15.0 | 5.0 | 12.5 | 10.9 |
| BHC | 1 lb./gal. (gamma) E.C. | 1 | 19.8 | 4.2 | 19.7 | 13.4 | 7.9 | 11.8 | 8.3 |
| Malathion | 5 lb./gal. E.C. | 1[c] | 17.0 | 7.3 | 25.8 | 9.6* | 5.2 | 8.3* | 8.6 |
| BHC | 1 lb./gal. (gamma) E.C. | 1[c] | 23.0 | 6.4 | 19.3 | 17.5 | 5.6 | 14.8 | 5.9 |
| Malathion | 5 lb./gal. E.C. | 1[d] | 14.2 | 5.4 | 16.4 | 7.4* | 6.2 | 12.6 | 9.6 |
| BHC | 1 lb./gal. (gamma) E.C. | 1[d] | 16.9 | 8.2 | 19.7 | 14.7 | 2.0* | 15.0 | 4.5* |
| Check | | | 18.5 | 8.3 | 22.4 | 19.9 | 8.0 | 19.9 | 9.8 |
| Difference required for significance at the 5% level[e] | | | 9.79 | n.s. | n.s. | 9.36 | 5.64 | 8.43 | 5.11 |

* Differs from check at the 5% level of significance (Duncan 1955).

[a] (ADJ) Mesick Plots. Spray dates adjusted on basis of saesonal insect development.

[b] Mean of counts on single tree plots, replicated 10 times. Counts represent product of number of infested buds (out of 50 per tree) and number of live larvae per infested 2-inch shoot tip (5 tips dissected per tree).

[c] Plus 1 lb. per 100 gallons of Aroclor 5460 (Havachlor Extender), supplied by Haviland Agricultural Chemical Company.

[d] Plus 5 lb., per 100 gallons, of proprietary spreader-sticker supplied by the Armour Chemical Division, Armour and Company.

[e] Computed from the maximum range of means using Duncan's (1955) multiple range table.

Lansing and Mesick, Michigan, in the summer of 1959. Applications made at the two locations are integrated chronologically on the basis of the insect's biology (see "Timing of Insecticide Applications").

Seven replicated experiments are shown along with column headings specifying the date when each treatment was made. Applications made on the same day have been subjected to analysis of variance. Means of each treatment were compared using the 5% significance table presented by Duncan in 1955. All materials were applied at the same concentration and in the same manner on each of the seven dates. With one exception, trees treated with dimethoate and DDT had significantly fewer live larvae if the treatments were made on or after June 30. The DDT exception, however, was significant at slightly over the 5% protection level used.

Applications of Sevin made on July 2, July 6, and July 9 all gave reductions in population that differed significantly from those noted on untreated check trees. Also, the DDT application made on June 12 reduced the shoot moth population considerably. This appears inconsistent, although an explanation may be sought on the basis of repellency of the spray mixture; toxicity of mixture to adult moths, or experimental error. (Reference to figure 1 "pupal cases in 100 infested shoots" shows that moth flight was approaching its peak at this time.)

Neither malathion nor BHC at the 1-lb. rate of application gave reductions in population that differed significantly from the check. However, such differences were observed where BHC plus Armour sticker was used on July 2 and July 9. Malathion plus Aroclor 5460 gave significant reduction of population in the June 30 and July 6 treatments, whereas malathion plus Armour evidently did the same on June 30.

DDT at the rate of 2 pounds actual toxicant per 100 gallons is the recommended insecticide for European pine shoot moth control in Michigan (Janes *et al.* 1958). Even at one-half of the recommended dosage it was comparable in these experiments to dimethoate and Sevin at 2 pounds per 100 gallons. Malathion, which was one of the most effective materials in dorsal dermal contact studies (Haynes 1959), did not compare favorably with DDT, dimethoate, or Sevin in these studies. Stickers improved the performance of malathion in several instances, and this fact suggests that residue persistence and/or ingestion of residue is more important in field control than dorsal dermal contact action.

Dimethoate has given good control to shoot moth in earlier experiments (Butcher & Haynes 1959) but residual (and perhaps foliar systemic) action could account for much of its success in field trials. BHC has not proved effective either in dorsal dermal contact experiments or under field conditions. Performance of this insecticide may be improved by addition of stickers, and mortality may be due to ingestion of residues, or penetration of ventral body areas, or both.

Superimposed on figure 1 are symbols representing applications of dimethoate, DDT, and Sevin made during the period of this study, June 12 to July 9, 1959. For each date, the vertical positioning of the symbol corresponds to per cent population reduction (from check) which resulted from its field application at that time. The obvious improvement in performance of these materials as the season progressed is seen in relation to biological and behavioral phenomena. It would appear that applications of each of these insecticides should be deferred until all of the adults have emerged and hatching is at least 50% complete.

The movement of newly hatched larvae to the needles and thence to the bud areas appears to be the single most important behavioral phenomenon operating in favor of control by residual type insecticides. Growers have long felt that it was necessary to make two applications of DDT, the second 10 days or 2 weeks after the first, in order to get effective control of shoot moth in the summer. The data presented in this paper provide evidence that the first application might have been premature in most

cases. The same result could be obtained by closer attention to insect development and the use of a single spray at the proper time.

CONCLUSION.—A number of insecticides and insecticide-sticker combinations reduce European pine shoot moth populations significantly. Either spring or summer applications may be successful if made at the proper time. The method of application used here is impractical under most Christmas-tree farming or forestry conditions where fast, economical coverage is necessary. Therefore, we haven't speculated upon possible success of any of these materials in low gallonage or aircraft spray application equipment. The problem of obtaining control where only low gallonage application equipment can be used economically remains. Nevertheless, the results of these efforts at relating insect biology and behavior to insecticide performance should help make it possible to apply future low gallonage sprays at a time when the insect is most vulnerable.

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# Control of the Grape Mealybug on Apricots[1]

HAROLD F. MADSEN[2] and LESTER B. MCNELLY[3]

ABSTRACT

The grape mealybug, *Pseudococcus maritimus* (Ehrh.), is a relatively new pest of apricots. This pest damages the fruit by colonizing in the stem end. The honeydew produced by the insects discolors the fruit and cannot be removed. The mealybug overwinters as crawlers within egg masses laid in cracks in the bark or in other protected places. The crawlers emerge in early spring and feed at the base of new shoots. A second generation develops by June, and crawlers of this generation infest the fruit. After harvest, mature females lay eggs that hatch by late fall. The young mealybugs remain within these cottony egg masses during the winter.

Experimental plots during the 1957–58 season showed that treatments timed to the spring emergence of crawlers were effective and were preferred to fall or winter sprays. Of the materials tested, parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) provided the best control. In 1959 pink bud and petal fall sprays gave better control than those applied during June, by which time some fruits were already infested. As in 1958, parathion and Diazinon were the most effective insecticides.

The grape mealybug, *Pseudococcus maritimus* (Ehrh.), is a relatively new pest of apricots, although it has been a common problem on other crops in California. The mealybug has a wide host range, and Essig (1958) lists a large number of both native and cultivated plants as hosts. Smith & Stafford (1955) state that the mealybug can cause severe damage to grapes. Madsen & Barnes (1959) list it as a major pest of pears.

Grape mealybug had not been reported on apricots in California prior to 1956. At the close of the harvest season, reports were received of damage to apricot fruits (fig. 1). Investigation showed that *P. maritimus* was involved. Studies were conducted on the biology and control of the mealybug from the 1957 season through 1959.

LIFE HISTORY.— The biology of grape mealybug on apricots is similar to that reported by Doutt & Hagen (1950) on pear. The insects overwinter as crawlers within white cottony egg masses deposited by the adult females during the fall. The egg masses are found in cracks or depressions in the bark of the trunk and main limbs (fig. 2). The crawlers become active shortly after the tree blooms in March and are commonly found at the base of the new shoots. They apparently feed on the tender tissue in this area and remain at the base of these shoots during early spring. They are not commonly found on the leaves, and

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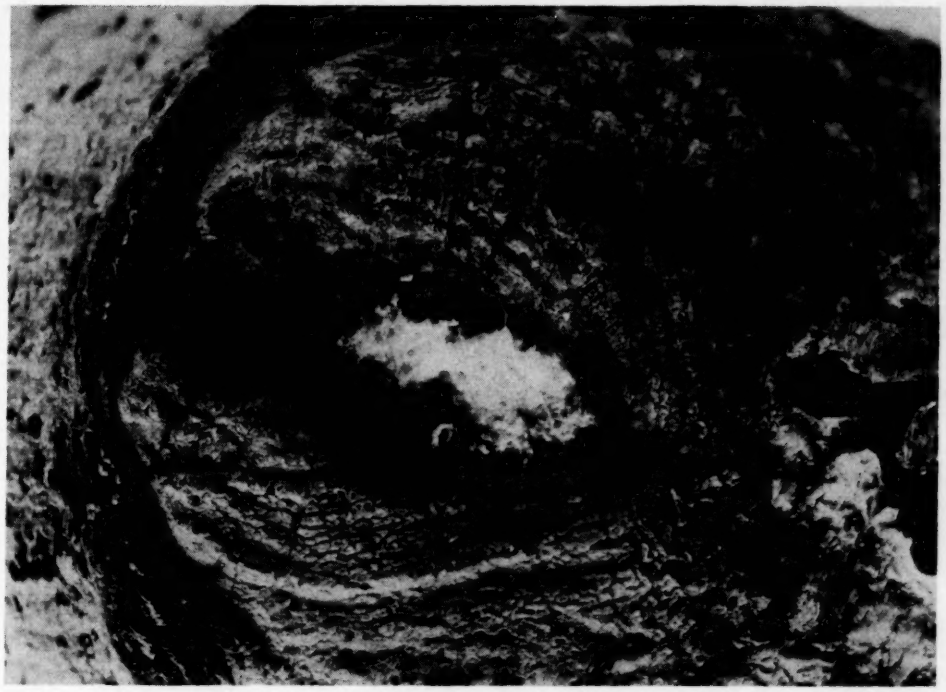

FIG. 1.—Overwintering colony of grape mealybug.

remain close to the limbs where they find protection beneath the bark scales. The crawlers reach maturity by late May, and the adult females of the new brood lay eggs in the cracks of the bark. These eggs hatch during June and the resulting crawlers move to the fruit or to the base of shoots. On the fruit they colonize in the stem end when the fruit grows large enough to be appressed to the twig. The crawlers mature in August after the fruit has been harvested. Adult females again deposit eggs in protected areas in the bark, and the eggs hatch by September or early October. The immature mealybugs remain within the old egg masses and overwinter as crawlers.

The principal damage caused by the grape mealybug on apricots is to the fruit. When the mealybugs colonize in the stem end of a fruit, they produce considerable honeydew, which runs over the side of the apricot. The black smut fungus that grows in the honeydew gives the fruit an unsightly appearance. In addition, the apricot skin seems to react to the presence of honeydew, as a red area develops beneath and bordering the deposit of honeydew. Fruit thus affected is not suitable for fresh shipment, and processors of unpeeled halves consider the contaminated fruit as culls.

CONTROL EXPERIMENTS—1957–58.—An apricot orchard with a history of mealybug attack was selected for the experimental plots. Trials were begun in the fall of 1957 and continued through 1958. It was decided to compare fall, winter, and spring treatments, because Stafford & Kido (1955) reported that dormant and summer sprays of parathion gave effective control on grapes. Frick & Bry (1955) also stated that both delayed dormant and summer sprays of parathion gave good control.

In addition to the different timing of treatments, several organic phosphates were evaluated as to their effect on mealybugs. The portion of the orchard used for the trials was divided into three sections. One received a fall spray, one a winter, and the third was treated during the spring. The fall and winter sprays were directed against the overwintering crawlers, and the spring spray was applied to coincide with the emergence of the crawlers from their overwintering sites.

The fall treatments were applied on September 15, using four phosphate compounds, each in combination with light-medium summer oil. The oil was added in the hope that it would improve the wetting and penetration

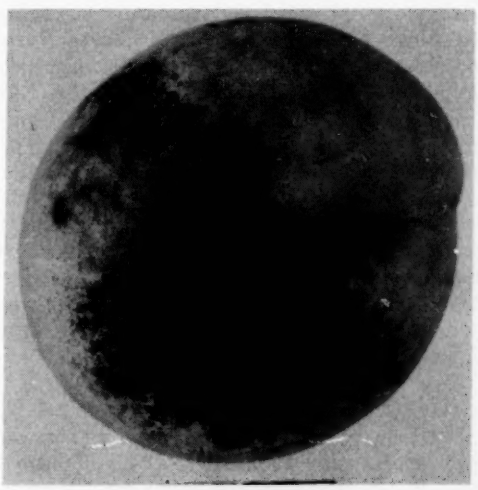

FIG. 2.—Apricot damaged by grape mealybug.

**Table 1.—Gilmeister Orchard, Cupertino, California. Summary of 1957–58 fall, winter, and spring plots for the control of mealybugs on apricots.**

| Material | Dosage per 100 Gal. | Reduction of Crawlers (%) | | Infested Fruit at Harvest on June 26, 1958 (%) |
|---|---|---|---|---|
| | | Oct. 17, 1957 | Jan. 28, 1958 | |
| Light medium oil+malathion,[a] 50% | 2 gal.+0.5 pt. | 26.1 | — | 10.2 |
| Light medium oil+trithion-EK,[a] 50% | 2 gal.+0.5 pt. | 80.0 | — | 7.0 |
| Light medium oil+phostex[a] | 2 gal.+1 pt. 8.0 conc. | 60.6 | — | 6.9 |
| Light medium oil+parathion,[a] 25% | 2 gal.+1 lb. | 82.1 | — | 4.6 |
| Check | None | — | — | 15.7 |
| Dormant oil+diazinon,[b] 25% | 2 gal.+1 lb. | — | 85.8 | 1.3 |
| Dormant oil+trithion-EK[b] | 2 gal.+0.5 pt. | — | 65.8 | 3.3 |
| Dormant oil+phostex[b] | 2 gal.+1 pt. 8.0 conc. | — | 50.5 | 5.6 |
| Dormant oil+parathion,[b] 25% | 2 gal.+1 lb. | — | 81.1 | 1.6 |
| Check | None | — | — | 13.0 |
| Sevin[c] wp, 50% | 1.5 lb. | — | — | 7.1 |
| Trithion[c] flowable, 50% | 0.5 pt. | — | — | 4.3 |
| Diazinon[c] wp, 25% | 1 lb. | — | — | 2.0 |
| Parathion[c] wp, 25% | 1 lb. | — | — | 0.6 |
| Check | None | — | — | 14.0 |

[a] Applied September 17, 1957.
[b] Applied January 7, 1958.
[c] Applied March 27, 1958.

of the spray. Each material was applied to four trees with three replications in a randomized block plot design. Sprays were applied with a conventional high-pressure spray rig and hand spray guns. An average of 550 gallons was applied per acre.

The same plot design and equipment was used for the winter and spring treatments. The organic phosphate compounds used during the winter were in combination with dormant oil. An average of 600 gallons per acre was applied at this time. The spring treatments were without oil, and the gallonage per acre averaged 650.

An attempt was made to determine the mortality of the overwintering crawlers following the fall and winter sprays. The cottony egg sacs were cut out of the bark and examined under a binocular microscope for living and dead mealybugs. This method did not prove very feasible as the number of living crawlers depended more upon the location of the colony than any other factor. Those colonies that were well hidden and deep within the bark had a high percentage of live crawlers, and those less protected showed a higher kill. It was possible, however, to eliminate compounds having little effect against this mealybug by this method. For example, malathion in the fall spray gave such a poor kill that it was not used during the winter or spring.

The final evaluation of the treatments and the best time to apply them was made during the 1958 harvest season. Six hundred apricots were examined per treatment. The fruit was picked at random from the trees. Fruits with mealybugs present or those showing honeydew and black fungus were recorded as infested.

The materials used, dosages, crawler, and fruit counts are summarized in table 1.

Of the compounds used, parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) provided the best control, regardless of timing of treatments. Trithion® (*S*-(*p*-chlorophenylthio)-methyl *O,O*-diethyl phosphorodithioate), malathion, Phostex® (a mixture of bis(dialkyloxyphosphinothiol)disulfides) and Sevin® (1-naphthyl-*N*-methylcarbamate), although reducing the mealybug damage below that of the untreated checks, were not so effective as these two compounds.

The winter sprays were superior to those in the fall, and this may be due to a bark residue, as has been pointed out by Stafford & Kido (1955). Although there was little difference between the two effective materials, Diazinon and parathion, in the winter and spring sprays, it is felt that spring would be the preferred timing. The difficulty in making contact with the well-protected colonies during the winter, and the added possibility of controlling other pests in the early spring give preference to this time of application.

Control Experiments 1959.—The results obtained in the 1957–58 trials indicated that control directed against the overwintering stages was not so effective as an early spring treatment. Since it is a common practice on apricots to apply a fungicide at the pink bud stage, and to start insect control measures at the petal fall stage, the 1959 plots were timed to these practices. As previously discussed, the overwintering crawlers emerge shortly after bloom and begin to feed on the new shoots. In addition, a summer spray was scheduled to be applied to coincide with the development of second-brood mealybugs.

The orchard selected for the trials had reported a mealybug infestation during the 1958 season, and winter observations showed the presence of egg sacs in the cracks of the bark. The portion of the orchard used for the trials was divided into three sections; one to receive a pink bud spray, one a petal fall, and the third a summer spray. Diazinon and parathion, which had given good control in the previous trials, were selected as the test insecticides. Ethion was also included and was formulated in a light summer oil containing 0.17 pound of actual ethion per gallon.

Within each section the plots consisted of single trees with five replications in a randomized plot design. Materials were applied with a conventional high pressure spray rig and hand guns. The gallonage per acre varied

Table 2.—Summary of 1959 mealybug plots on apricots, Johnson Ranch, Cupertino, California.

| Material | Dosage per 100 Gallons | Per Cent Infested Fruit[a] | | |
|---|---|---|---|---|
| | | Pink Bud Sprays[b] | Petal Fall Sprays[c] | Summer Sprays[d] |
| Parathion, wp | 1 lb. 25% | 0.9 | 0.0 | 1.0 |
| Ethion-oil, ec | 2 gal. | 3.7 | 1.5 | 5.4 |
| Diazinon, wp | 1 lb. 25% | 0.4 | 0.1 | 1.2 |
| Ethion, wp[e] | 1 lb. 25% | — | 1.6 | — |
| Check 1 | Unsprayed | 6.9 | — | — |
| Check 2 | Unsprayed | — | 4.2 | — |
| Check 3 | Unsprayed | — | — | 9.5 |

[a] Harvest counts made June 30.
[b] Applied March 2.
[c] Applied March 26.
[d] Applied June 5.
[e] Applied only at petal fall period.

from 450 at the pink bud spray to 600 during the foliage season. Dodine® (also designated Cyprex: *n*-Dodecylguanidine acetate) was added to the insecticides at the pink bud and petal fall stages for control of brown rot. In early June, observations showed that the second brood of crawlers was beginning to emerge. The summer spray was therefore applied on June 5.

At harvest, fruits were picked at random from the trees in the plot and were examined for mealybugs or mealybug damage. Two hundred fruits were checked on each replicate—a total of 1,000 per treatment. The materials used, dosages, and counts of fruit at harvest are summarized in table 2.

Diazinon and parathion, as in 1958, gave comparable control at the three periods of application tested. Ethion did not provide as satisfactory control. At the petal fall stage, the oil-ethion combination was no better than the wettable formulation.

Good control of the mealybugs was obtained at either the pink bud or petal fall stage treatments. The June sprays were not so good, probably because at this time some mealybugs are already hidden in the stem end of the fruits. An overlap occurs between first and second broods. It would, therefore, be very difficult to time the summer sprays so that crawlers would be controlled before they damaged the fruit.

Although the pink bud and petal fall treatments were comparable in this trial, it should be pointed out that the winter and early spring of 1959 was one of the warmest seasons on record. It may be possible that crawlers would not emerge from the overwintering sites as early in a season with normally cool temperatures. The Dodine used at the petal fall stage caused leaf mottle and a light discoloration of the fruit, which was barely discernible at harvest. When combined with the insecticides, this effect was intensified, especially when combined with ethion and ethion-oil.

The experimental plots over the 2-year span have led to the suggested use of either parathion or Diazinon for mealybug control. The petal fall stage is the preferred time of application, as it is possible to obtain added benefit from these two compounds on other apricot pests that are active at this time.

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# The Effects of DDT and Malathion upon Forest Soil Microarthropods[1]

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Abstract

Throughout a one-year period a study was conducted to determine the effects of plot treatments of DDT and malathion on beneficial forest soil mites and Collembola. Twenty-four plots were established in each of two habitats, a red pine plantation and a mixed hardwood stand. Six different treatments were replicated four times in each habitat. Two collections before treatment and seven collections after treatment in each habitat over a one-year period resulted in data to which an analysis of variance could be applied. The application of DDT at concentrations used 10 times in excess of that used in current forest pest control programs and malathion used at the rate of 2 pounds per acre are without harm to the organisms studied.

Few studies have been conducted to determine the effects of insecticides upon soil microarthropods. Furthermore, where such studies have been made, large areas have been sampled to obtain general information. Hoffmann & Merkel (1948) found mites unaffected in

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forest soil that had been treated with DDT at the rate of 4 lb. per acre, although sampling had been carried out in the center of 100- to 600-acre tracts within large forest areas that had been sprayed from the air. Sheals (1955) found increases in Collembola populations and a reduction in mites over a 1-year period in plots treated with DDT at the rate of 13 lb. per acre. In these, and in other cases reviewed by Satchell (1955), either large tracts of land were sampled extensively or inadequately or the changes before and after treatment were not accompanied by adequate statistical analyses.

There is a need for intensive studies of the effects of insecticides upon soil microarthropods. These organisms are extremely beneficial to forest soils. Owing to their tunneling activities the aeration and moisture retention properties of the soil are improved. Through the role they play in decomposing organic matter and depositing feces and their own bodily elements after death, the structure and fertility of the soil are maintained. The number of these organisms harbored by a unit area of forest soil is indeed great. Wallwork (1957) estimated 2.35 mites per cc. in a hemlock forest. In the same volume of soil, Evans (1955) estimated 2.5 mites in a spruce plantation. The data of Macfayden (1952) indicated approximately two mites per cc. and one Collembola per 5 cc. in a fen. The beneficial activity of these organisms as mentioned above is important in view of these large numbers.

Since it is known that DDT and certain other insecticides accumulate in the soil with repeated spraying, it is necessary to consider the effects of these accumulations upon the fauna. This study was conducted to determine the effects of DDT and malathion upon soil microarthropods. The study was made intensive through the use of known quantities of the toxicants and through the use of intensive sampling before and after treatment. Both qualitative and quantitative effects were taken into consideration.

EXPERIMENTAL METHODS.—Experimental work was done in 1958 in a plantation of red pine (*Pinus resinosa* Ait.) and in a mixed hardwood stand in the Tully Forest located 20 air miles south of Syracuse, New York. Both stands were relatively uniform in appearance and were on level land. The pine plantation was a 23-year-old stand planted in rows at intervals of 6 ft. The forest floor was covered with a 1- to 2-inch blanket of pine needles. The mixed hardwood stand was unevenly aged, with an understory consisting of several species of grasses, ferns, and seasonal wild flowering perennials common to the Northeast.

The soil in the mixed hardwood stand belongs to the Lordstown series (Cline 1957). This series is described as a shallow well-drained, moderate podzolic soil that is very low in lime. The parent material is a glacial till, predominantly shale and sandstone. The soil in the red pine plantation is described as a moderate podzol with a fragipan. It belongs to the Mardin series (Cline 1957) and is also of glacial till origin, predominantly of shale and sandstone and very low in lime.

Twenty-four 10.5-ft. square plots were established at random over level ground in each habitat. In making a collection, 12 cores, each measuring three-fourths inch in diameter and 3 inches deep were removed from each plot.

Prior to treatment, two collections were made at each habitat to determine the variance "between" replication-series and the variance "within" replication-series. A replication-series is a set of four plots that received similar treatment. The four plots were assigned one of six treatments at random, using a table of random numbers.

All plots except the controls were sprayed with a Hudson portable sprayer operated at a pressure of 60 lb. p.s.i. The six treatments used are listed below along with the reason for their choice.

1. *Control.*—The numbers and species of animals found on these plots furnished a basis for determining the changes in animal populations as affected by the chemical treatment.
2. *Diluent.*—Plots were treated with the solvent Sovicide[3] which served as a vehicle for carrying DDT and malathion. This diluent was used to determine whether it had any effect on soil fauna. Since it did not, these plots also served as control plots for comparison.
3. *DDT[4] at 1 lb. per acre.*—This is the concentration of DDT used in current forest pest control programs.
4. *DDT at 10 lb. per acre.*—This concentration of DDT was used because it is known that DDT is a residual toxicant.
5. *DDT at 50 lb. per acre.*—This high concentration of DDT was used in order to determine to what extent it would be a population density reducing factor.
6. *Malathion[5] at 2 lb. per acre.*—This insecticide was used primarily because it possessed acaricidal properties.

In sampling the soil, an 8- by 11-inch sheet of paper was placed upon the forest floor. The cores were removed by placing the core-sampling tool into the soil at sites chosen at random while traversing the plot in a circular direction. Each core ($3'' \times \frac{3}{4}''$ diameter) was gently transferred from the sampling tool to the paper. After removing 12 cores from a plot, the sample was transferred to a plastic bag and transported to the laboratory.

The microarthropods were extracted from the soil through Berlese funnels (Macfayden 1953). Approximately 95% of these soil microarthropods were mites and Collembola. The remaining organisms classified in this study as microarthropods included only those arthropods capable of passing through a screen that had 16 meshes to the inch. This included pseudoscorpions, spiders, millipedes, centipedes, and larval and adult forms of insects.

The effectiveness of the Berlese extraction process was shown by Wallwork (1957) to be between 85% and 100%. Van Der Drift (1951), using a different checking system, claimed a recovery of from 80% to 100% for most of the organisms studied. No attempt was made in this study to check the extraction efficiency. Where comparative quantitative studies are being pursued, the percentage recovery is not so important as an accurate constant proportion recovery from each funnel for each collection date.

The counting of the extracted organisms was done with the aid of a gridded counting plate placed under a 6-inch saucer. The grids allowed coverage of the entire saucer area. Mites and Collembola collected in this study are listed in Hartenstein (1959).

RESULTS.—A study of the distribution of individual species, genera, families, suborders, and other groups along with the main theme of this study was impractical.

[3] Sovicide 544 B, Standard Oil Company, Chicago 80, Illinois.
[4] Technical, Monsanto, setting point 89 degrees.
[5] Technical, American Cyanamid Company.

Table 1.—Analyses of variance—Collections 1 to 18.

| Collection | Mean Square: Within Replication-Series | Mean Square: Between Replication-Series | F |
|---|---|---|---|
| | *Before treatment* | | |
| 1 | 2.0 | 0.8 | 0.4 |
| 2 | 0.5 | 0.5 | 1.0 |
| 3 | 3.8 | 3.1 | 0.9 |
| 4 | 1.7 | 2.2 | 1.3 |
| | *After treatment* | | |
| 5 | 6.2 | 2.2 | 0.3 |
| 6 | 2.9 | 3.8 | 1.3 |
| 7 | 17.0 | 10.0 | 0.6 |
| 8 | 1.4 | 10.0 | 6.6[b] |
| 9 | 9.2 | 11.0 | 1.2 |
| 10 | 2.0 | 2.0 | 1.0 |
| 11 | 2.7 | 87.0 | 32.0[b] |
| 12 | 0.7 | 2.0 | 2.9[a] |
| 13 | 13.0 | 49.0 | 3.8[a] |
| 14 | 2.8 | 2.4 | 0.9 |
| 15 | 6.3 | 19.6 | 3.1[a] |
| 16 | 1.0 | 22.0 | 22.0[b] |
| 17 | 5.4 | 6.7 | 1.2 |
| 18 | 1.3 | 2.3 | 1.7 |

$F_{0.05}$2.7 and $F_{0.01}$4.25 where $F_1 = 5$ and $F_1 = 18$.
[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.

It was therefore unknown whether the data of the study were distributed at random and therefore Poisson in nature, or whether the data were contagiously distributed. Large differences between the means and the ranges for the six replication-series of the two pretreatment collections in each of the two habitat types suggested heterogeneous variance. Distribution curves for the 24 total counts in each of the four collections were skewed to the right. Beall (1954) stated that a transformation of entomological data to their square roots is often effective in normalizing the data. An arrangement of the transformed data into frequency distribution curves for the collections mentioned above indicated considerably less skew. The coefficients of variation of the transformed data for each of the collections were reduced by 50%. The transformation of the data made it amenable to an analysis of variance. Analyses of variance for every collection (table 1) were used to determine whether the difference in the means for the total microarthropods in a replication-series were significant.

Four conditions must be satisfied before employing the analysis of variance. First, the observations (total microarthropods in each sample) must be normally distributed. This condition was partially met by using the square-root transformation as has been indicated above. Slight abnormality may weaken the analysis for detecting significant differences but does not impair its usefulness for detecting large significant differences. Second, the variance in the subpopulations (replication-series) taken from the parent population and the variance between the subpopulations must be the same before treatment. Any differences in these variances must be accountable to random sampling. Bartlett's test of homogeneity of variance (Snedecor 1956) was applied and indicated uniformity of variance in all pretreatment collections. The third condition is that the mean for each subpopulation (replication-series) must be approximately the same before treatment. This condition was met through the analyses of variance (table 1). The data of the analyses indicate a probability which is higher than 25% of a greater F in each of the pretreatment collections—a probability well within the region of nonsignificance. Finally, the members of each subpopulation must be drawn at random from the parent population. This condition was met by assigning treatments to the plots using a table of random numbers.

Throughout the ensuing presentation of the results the reader should refer to figures 1 and 2 and to table 1. The figures indicate the trends in population changes both before and after treatment as well as the period between collections. Though the graphs show changes for the total community of microarthropods only, the changes are similar to those occurring in the mite population and in the Collembola population (Hartenstein 1959).

The analysis of the pretreatment data indicated similar population behavior in all replication-series for both habitats. The populations increased in all of the replication-series from the first pretreatment collection to that of the second.

*The Effects of Treatment in the Mixed Hardwood Stand.*—The population of microarthropods in each replication-series increased or decreased simultaneously during the first 4 weeks after treatment. A study of the data and figure 1 suggested that the differences between the means for each replication-series in Collections 5, 7, and 9 were due to natural causes. The analyses of variance confirmed this premise (table 1). Thus, during the first 4 weeks after treatment, any changes in population structure were not or could not be detected. Nor could any changes be ascribed to a particular treatment effect.

The population of microarthropods decreased in all replication-series 8 weeks after treatment (Collection 11, fig. 1). The analysis of variance showed that the differences between the means were the results of the different treatments. Tukey's test (Snedecor 1956) was used to determine which differences were significant. Significant differences were found between the means for the replication-series treated with 10 lb. per acre of DDT and those of all other replication-series except that treated with DDT at the rate of 1 lb. per acre. Significant differences also were found between the mean for the replication-series treated with 50 lb. per acre of DDT and the mean for those treated with the diluent and the controls.

Fifteen weeks after treatment the population density increased in the replication-series treated with DDT at the rates of 10 or 50 lb. per acre. The increases were unusually large in comparison with densities in the other replication-series. The analysis of variance revealed significant differences between the means. Tukey's test showed that the differences existed between the means for the replication-series treated with DDT at the rates of 10 or 50 lb. per acre and the means for all other replication-series.

Many other investigators have reported increases of certain populations exposed to insecticides. Sheals (1955) reported increases in Collembola populations exposed to DDT treatment. Steiner *et al.* (1944) noted increases in

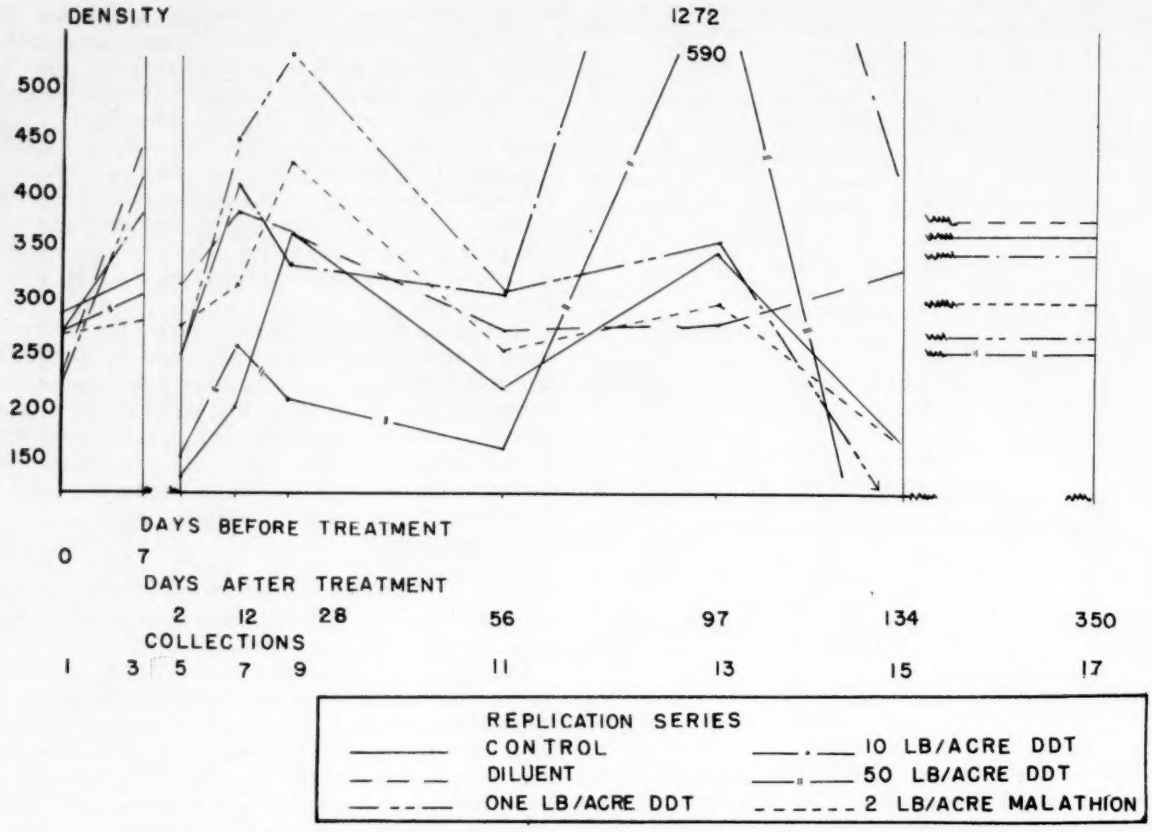


FIG. 1.—Total number of microarthropods from each replication-series for each collection in the mixed hardwood stand.

foliage mites that had been exposed to DDT treatment. Similar reports have been made by Hough (1946), Newcomer & Dean (1946), Dean (1950), Klostermeyer & Rasmussen (1953) and others. The mechanism for population increase could not be revealed in any of these studies or in the present study.

There was a reduction in the microarthropod population in all replication-series except for the one treated with the diluent 19 weeks after treatment. An analysis of variance again indicated a significant F value.

A collection taken 1 year after treatment revealed no significant differences between treatments. The trends in mite and Collembola populations paralleled the trends in the total microarthropod population throughout the entire study period. In summary, the use of DDT at 1 lb. per acre and malathion at 2 lb. per acre and the use of Sovicide did not result in any population behavior different from that in the untreated control plots. DDT at the rates of 10 or 50 lb. per acre did result in population changes but the changes were short lived.

*The Effects of Treatment in the Red Pine Plantation.*—The use of DDT at the rates of 1 and 10 lb. per acre, malathion at 2 lb. per acre, and the use of Sovicide did not result in any population changes that differed from those in the control plots throughout the duration of the study. In the plots treated with 50 lb. per acre of DDT the microarthropod population was reduced throughout the posttreatment period (fig. 2). The analyses of variance for the counts made from collections taken in this stand revealed significant differences among the means for Collections 8, 12, and 16. The significant differences always were detected between the mean for the replication-series treated with 50 lb. per acre of DDT and those of four or five other replication-series. Thus microarthropods were reduced in the heavily treated plots, which fact could not be attributed to sampling variation. This reduction can be shown in another way. In pretreatment sampling, the microarthropod numbers were higher in the replication-series subsequently treated with 50 lb. per acre of DDT than in any other replication-series except the one which was later treated with DDT at the rate of 10 lb. per acre (fig. 2). After treatment, the microarthropod density in the replication-series treated with 50 lb. per acre of DDT was considerably lower than the density in any other replication-series in all collections but one.

One year after treatment, the population density in the plots treated with 50 lb. per acre of DDT was lower than in any other replication-series although the difference between its mean and the mean of any other replication-series was not significant.

*The Effects of Treatment upon Subdivisions of the Microarthropod Population.*—In the planning of the study and in the analysis of the data, consideration was given to the fact that some niches and microhabitats might become vacant following chemical treatment and that these niches and microhabitats could be occupied by other organisms

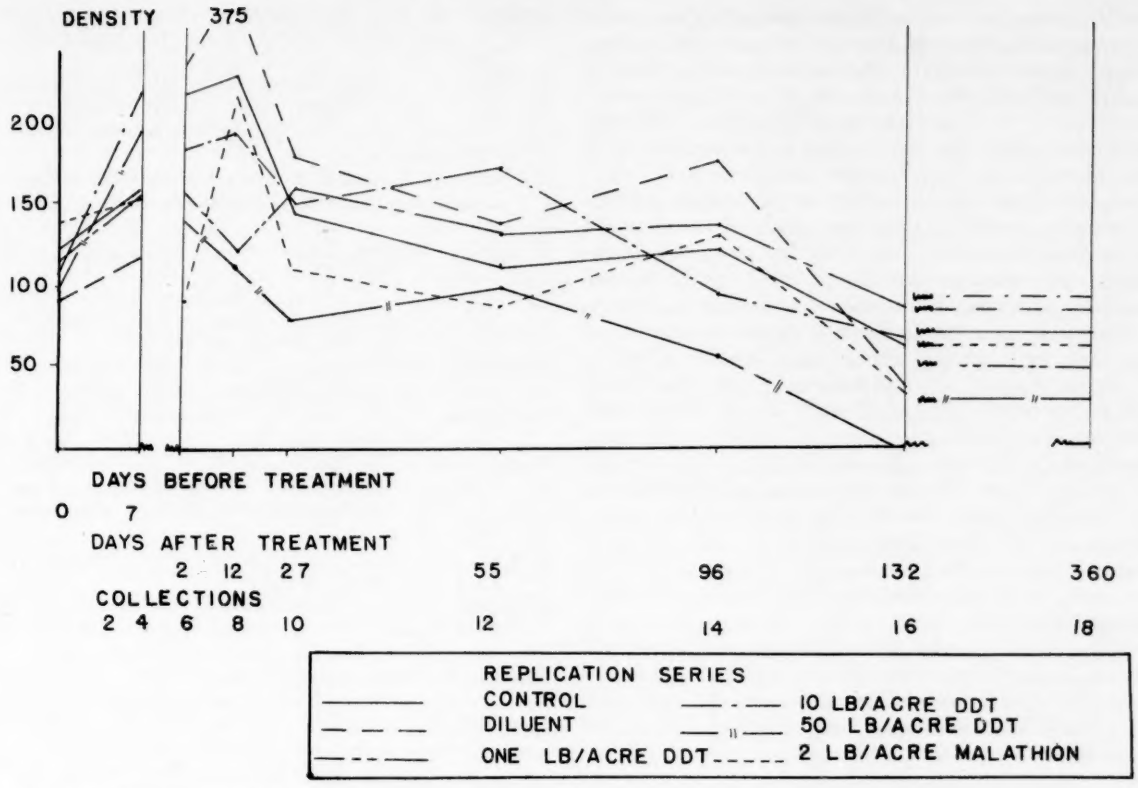


FIG. 2.—Total number of microarthropods from each replication-series for each collection in the red pine plantation.

less susceptible to the chemicals. Therefore, the trends in total numbers of microarthropods would not indicate any of the internal changes that might have taken place in the species organization of the community. The mite population was the only group that could be separated because it was the only group of microarthropods the subdivisions of which had sufficient numbers of organisms for analysis. Three subdivisions of mites were recognized, these being the oribatids, mesostigmatids, and juveniles.

A close examination of all data revealed two types of internal changes. First, the juvenile mite subdivision increased or decreased concomitantly with an increase or decrease, respectively, in the mite population. This subdivision, which included both mesostigmatids and oribatids, duplicated the trends manifested in the mite population. It formed a representative sample of the mite population. This behavior did not allow any distinctions between treatment effects upon juveniles and the adult mites. Second, the oribatids and mesostigmatids increased or decreased concurrently or one of the two subdivisions increased while the other was reduced. The increase in one subdivision with a concurrent decrease in the other suggested a predator and prey relationship, the mesostigmatids preying upon the oribatids. However, close scrutiny of all the data showed that an increase in one group with a decrease in the other occurred with equal frequency to concomitant increases or decreases in both groups.

DISCUSSION.—There is no longer any question regarding the importance of the use of insecticides as a forest conservation measure. Without the advances made in organic insecticide research and production, many merchantable stands of timber would become pest infested and the quality and value of such stands would be reduced if not lost. However, it is necessary to guard against unnecessary use of these poisons since in addition to the phenomenon of insect resistance after repeated use of a certain kind of insecticide, pest increase becomes an associated danger when the pest either has become resistant to the toxicant or lacks susceptibility to the particular poison used.

Fortunately, the use of DDT at rates currently used in control programs and malathion at rates slightly higher than those used in practice are ineffective in reducing or increasing beneficial soil microarthropods, *i.e.*, ineffective in upsetting a "balance of nature." Even at the high concentration of 10 lb. per acre applied directly to the soil so that there was no loss to foliage, DDT was ineffective in the red pine plantation where population densities are relatively low, and of ephemeral effect in the hardwoods stand, resulting in an increase in organism density.

Although the study as reported here does not furnish evidence to indicate why DDT failed to reduce the microarthropods, another investigation reported by the author (1959) did. In a bioassay, using *Musca domestica* L. as a test organism, the author showed that when DDT is mixed through 2 gm. of soil that was spread over a $1\frac{1}{4}$- by 4-inch strip of paper, five times as much toxicant was needed to produce 64% mortality as was needed when the same quantity of DDT was distributed as a dry film

over 1 sq. in. of wall surface on the inside of a glass bottle. This was because the soil possessed a larger surface area. When treated with DDT, the amount used per unit of surface area was reduced and some of the surface was inaccessible to the organisms. In addition, less DDT was available to the organisms during a specified period of time in the flasks containing the soil. Menn *et al.* (1957) have shown that flies exposed to lethal dosages of DDT for varying periods of time can recover if the exposure period is insufficient. It is possible that many microarthropods may come into contact with DDT crystals but the exposure period may be insufficient to produce mortality.

Malathion also was ineffective in regulating the soil microarthropod density. This toxicant is water soluble to the extent of 145 p.p.m. and it is easily hydrolyzed above pH 7.0 or below pH 5.0 (Metcalf 1955). Twenty-four samples of soil from the mixed hardwood stand, when measured, had a mean pH value of 5.1 with a standard deviation of 1.14. The same number of samples from the red pine plantation had a pH value of 4.8 with a standard deviation of 0.7. It is believed therefore that the many inches of rainfall that fell during the first month after treatment would have hydrolyzed all this organic phosphorus insecticide.

Although the posttreatment study was conducted within 1 year, the effects of the treatment extended through many life cycles. During this period innumerable mites and Collembola were born and had died within small areas.

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# Soil Treatments with Insecticides for Control of the Eye Gnats *Hippelates collusor* and *H. hermsi*[1]


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## Abstract

*Hippelates* gnats breed predominantly in loose, cultivated soils, and one approach to their control is through soil treatments. The effectiveness of aldrin and other soil insecticides was investigated in the field. Aldrin and heptachlor sprays and granules within the range of practical dosages produced moderate initial control of *Hippelates* gnats but gave no significant reduction in gnat breeding after a lapse of 7 to 10 months. Dieldrin was not tested for its initial effectiveness, but at moderate dosages gave no control of gnats 7 months after application. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at the highest applied dosage (6.2 lbs. per acre) was ineffective, and results with toxaphene were inconclusive.

DDT was the only insecticide among the materials tested that gave a high degree of initial control of *Hippelates collusor* (Townsend). This material, when applied at 13 pounds actual toxicant per acre, gave 95% reduction of the emerging gnats initially. The long-term residual effectiveness of this material is not yet known.


*Hippelates collusor* (Townsend) and *H. hermsi* Sabrosky breed predominantly in loose, cultivated soils. The adult gnats are attracted to freshly tilled ground and presumably lay eggs at or near the surface of the disturbed soil. On hatching, the larvae enter the soil and feed on decay-


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ing organic matter that has been turned under during disking. After completion of development, the larvae pupate primarily in a zone 2 to 6 inches below the soil surface. The emerging adults work their way up through the soil. The adults of *H. collusor* are a nuisance; they are severe pests in agricultural operations and infest residential and recreational areas as well.

Since the immature stages of the gnats develop in the soil, the possibility of treating the breeding grounds with residual insecticides to abate gnat populations was hopefully considered for some time. This means of control was not attempted, however, until the advent of chlorinated hydrocarbon insecticides. About 1951, this group of insecticides was employed for the first time in experimental control programs for *Hippelates* eye gnats in the Coachella Valley (Tinkham 1952, 1953). The results were interpreted as indicating that aldrin at 2 pounds actual toxicant per acre was superior to other chlorinated hydrocarbon materials applied at the same rate and to DDT applied at 6 to 22 pounds actual toxicant per acre. The aldrin treatment was believed to yield a high degree of control of eye gnats for 2 to 3 years. The major breeding grounds were therefore treated by the Coachella Valley Mosquito Abatement District about every third season during the period 1951 to 1956.

The present studies were undertaken during the 1956 gnat season to obtain more complete information on the larvicidal value of aldrin and to investigate the effectiveness of other residual soil treatments against eye gnats.

METHODS AND MATERIALS.—The insecticides under investigation were applied to the surface of the soil either as granules or as dilute sprays. The granulated formulations were applied with a twin rotor broadcaster (Mulla & Barnes 1958) calibrated to apply the desired quantity of material per acre. The sprays were applied with low-pressure boom sprayers at the rate of 50 to 60 gallons per acre.

Plots having weeds or cover crops were disked once prior to treatment, so that liquid materials could easily be applied directly to the surface of the soil without depositing an appreciable amount of the toxicant on plant foliage. In order to minimize the loss of insecticides from the soil surface, the plots were disked immediately after treatment. In some cases, the posttreatment disking operation took several hours, but in no case were the test plots disked later than the day they were treated. The depth of the disking ranged from 4 to 6 inches. In some instances, two posttreatment disking operations were required to turn the organic matter under thoroughly.

For the purpose of ascertaining gnat breeding, emergence cages were set in the test plots 10 to 20 days after posttreatment disking. During this period, the gnats completed their development in the soil and started to emerge. The emergence cages were 1-square-yard, 12-inch-deep frames covered on the top with black cloth and canvas. A quart jar ring was fitted into a circular hole near the top edge of each end of the cage. Quart jars fitted with 40-mesh strainer-cloth cones with an opening at the tip were fitted into the rings. The gnats emerging from the soil were attracted to light. They crawled through the screen cone into the collection jar, their re-entry into the cage restricted by the cones. The jars from these cages were collected twice a week, and the gnats were counted in the laboratory. A buffer zone of 20 to 30 feet was maintained between adjacent plots. The emergence cages remained in place for a period of 3 to 6 weeks. In summer, emergence of adult gnats was complete in about 3 weeks; in the fall, emergence took 5 to 6 weeks.

The data were analyzed by analysis of variance. The values were transformed to $\sqrt{X \times 1}$ where the numbers were small and to $\sqrt{X+0.5}$ where the values were relatively larger. The results of these statistical analyses are included in the tables.

RESULTS AND DISCUSSION.—The extent of breeding of eye gnats in cultivated ground was, of course, subject to a great deal of variation. The amount of organic matter and of moisture in the soil, variation in soil texture, and other factors influenced gnat breeding. These variables may be minimized by adequate replication and by increasing the number of emergence cages per plot, when possible. With this fact in mind, and to compare effects of materials under different circumstances to insure infestation, experiments were planned with the same materials under diverse conditions. Relatively high dosages were employed initially so that unpromising materials could be identified quickly.

The initial effectiveness of aldrin spray and granules, when applied at 3.6 or 3.8 pounds actual toxicant per acre, was relatively low, and no significant difference between the spray and the granules was observed (table 1). At the same time, heptachlor granules at 4.4 pounds actual toxicant per acre yielded about the same degree of control as aldrin granules. As demonstrated in laboratory trials (Mulla 1960), the initial effectiveness of aldrin and heptachlor at practicable dosages was low. Much higher dosages must be employed in order to obtain a high degree of control of *H. collusor*. Emergence data from Kersteiner's date-garden plots, 10 months after treatment, indicated no significant difference between the check and the aldrin and heptachlor treatments (table 1). The short-term duration of aldrin and heptachlor in the soil is probably caused by an appreciable conversion of these materials to their epoxides (Bollen *et al.* 1958, Gannon & Bigger 1958). Since the epoxide of aldrin (dieldrin) has been shown to manifest low initial biological activity against *H. collusor* in laboratory studies (Mulla 1960), it seems reasonable to conclude that the initial effectiveness of aldrin was lost as a result of this conversion process. Owing to the lower activity of dieldrin against *H. collusor*, the amount of dieldrin produced from a practical dosage of aldrin would be too small to produce an appreciable reduction of the emerging gnats.

Investigations carried on in another date garden on the effectiveness of aldrin and heptachlor sprays and granules as soil treatments against *Hippelates* gnats yielded results comparable to those obtained in the previous test (table 2). In this case, aldrin granules and sprays applied at 4.8 and 4 pounds of actual toxicant per acre, respectively, gave poor control of *H. collusor* and *H. hermsi* 9 months after treatment. Heptachlor granules and sprays applied at 5.3 and 3.8 pounds of actual toxicant per acre, respectively, also resulted in poor control or no control of the two species of gnats. The emergence of eye gnats after application of aldrin and heptachlor spray and granulated treatments was not significantly different from that of the checks after a 9-month interval.

**Table 1.—Initial and short-term effectiveness of aldrin and heptachlor against *Hippelates collusor* when applied to soil in the Kersteiner date garden. Indio, California.[a]**

| Treatment | | Pounds Toxicant per Acre | Average No. of Gnats/Cage and Reduction (%) | | | |
|---|---|---|---|---|---|---|
| | | | October 1956[b] | | July 1957[c] | |
| Material | Formulation | | Gnats/Cage | Reduction (%) | Gnats/Cage | Reduction (%) |
| Aldrin | Emulsifiable concentrate[d] | 3.6 | 4[e] | 53 | 14 | 0 |
| Aldrin | 10% on 30/40 LVM-A Attapulgite | 3.8 | 2[e] | 75 | 12 | 0 |
| Heptachlor | 10% on 30/60 LVM-A Attapulgite | 4.4 | 2[e] | 75 | 9 | 13 |
| Check | | | 9 | — | 10 | — |
| | | | | | N.S. | |

[a] Treated September 19, 1956. Each treatment was replicated three times at random, except the check which was replicated twice. Soil type: Coachella fine sand.
[b] Six 1-square-yard emergence cages were set up in each plot. Two replicates tested.
[c] Nine 1-square-yard emergence cages were set up in each plot. All replicates tested.
[d] Containing 3 pounds actual toxicant per gallon. A dilute spray was prepared from this and applied at the rate of 52 gallons per acre.
[e] Significantly different from check at 0.01 level.

**Table 2.—Short-term effectiveness of aldrin and heptachlor as soil treatments against *Hippelates* gnats in a date garden at Indio, California.[a]**

| Treatment | | Pounds Toxicant/Acre | Average No. of Gnats/Cage and Reduction[b] (%) | | | |
|---|---|---|---|---|---|---|
| | | | *H. collusor* | | *H. hermsi* | |
| Material | Formulation | | Gnats/Cage | Reduction (%) | Gnats/Cage | Reduction (%) |
| | | *A—Granules (two replicates)*[c] | | | | |
| Aldrin | 10% on 30/40 LVM-A Attapulgite | 4.8 | 55 | 9 | 20 | 0 |
| Heptachlor | 10% on 30/40 LVM-A Attapulgite | 5.3 | 32 | 47 | 10 | 0 |
| Check | | — | 60 | — | 9 | — |
| | | | n.s. | | n.s. | |
| | | *B—Sprays (one replicate)*[d] | | | | |
| Aldrin | EC 3[e] | 4.0 | 78 | 20 | 35 | 0 |
| Heptachlor | EC 2[e] | 3.8 | 95 | 2 | 20 | 0 |
| Check | | | 97 | — | 20 | — |
| | | | n.s. | | n.s. | |

[a] Treated in November 1956; adult emergence occurred in July 1957. Soil type: Indio very fine sandy loam.
[b] Ten 1-square-yard emergence cages were set up in each plot.
[c] Only two of the total three replicates could have been tested.
[d] Only one of the total four replicates could have been tested.
[e] Emulsifiable concentrates containing 3 and 2 pounds actual toxicant per gallon.

**Table 3.—Short-term effectiveness of aldrin, dieldrin, and heptachlor as soil treatments against *Hippelates collusor* in the Ebeling citrus grove, Indio, California.[a]**

| Treatment | | Pounds Toxicant/Acre | Average No. of Gnats/Cage[b] | Reduction (%) |
|---|---|---|---|---|
| Material | Formulation | | | |
| Aldrin | Emulsifiable concentrate | 5.5 | 60 | 18 |
| Aldrin | 10% on 20/30 Attapulgite granules | 8.0 | 69 | 6 |
| Dieldrin | 10% on 20/30 Attapulgite granules | 6.4 | 75 | 0 |
| Heptachlor | 10% on 20/30 Attapulgite granules | 7.0 | 51 | 30 |
| Check | | — | 73 | — |

[a] Soil treated November, 1956; adult emergence occurred in June 1957. Each treatment was replicated five times, except the check, which was replicated six times. The organic matter content turned under at the time of disking was high. Soil type: Coachella fine sand.
[b] Four to seven 1-square-yard emergence cages were set up in each plot and the differences were not significant.

A concurrent trial on the short-term effectiveness of aldrin sprays and granules and of heptachlor and dieldrin granules was carried out in a citrus grove where a larger quantity of organic matter was disked under. Owing to larger particle size of the granules, the dosage of the actual toxicant of aldrin for this formulation was increased over that of the spray application. The control of *H. collusor* obtained from these treatments after 7 months was very low (table 3). Gnat emergence from plots treated with aldrin spray and granules (applied at 5.5 and 8 pounds actual toxicant per acre, respectively), and with heptachlor and dieldrin granules (applied at 7 and 6.4 pounds actual toxicant per acre, respectively) was not significantly different from the untreated check.

The first series of trials established the ineffectiveness of aldrin, heptachlor, and dieldrin for gnat control; therefore, other materials had to be tried. In preliminary trial, results with toxaphene and DDT granules were compared with those obtained with aldrin granules. The initial effectiveness of toxaphene granules (applied at 9.7 pounds actual material per acre) was equal to that of aldrin (applied at 2.2 pounds actual toxicant per acre). DDT (at the rate of 13 pounds actual toxicant per acre) caused greater reduction of *H. collusor* in this trial than that of toxaphene

Table 4.—Initial effectiveness of aldrin, toxaphene, and DDT granules as soil treatments against *Hippelates collusor* in Foster young citrus grove, Indio, California.[a]

| Treatment | | Pounds Toxicant/ Acre | Average No. of Gnats/ Cage[b] | Reduction (%) |
|---|---|---|---|---|
| Material and Concentration | Type of Attapulgite Granules | | | |
| Aldrin, 10% | 30/60 LVM-A | 2.2 | 19 | 51 |
| Toxaphene, 10% | 30/40 RVM-AA | 9.7 | 11 | 72 |
| DDT, 10% | 30/40 RVM-AA | 13.0 | 2 | 95 |
| Check | | — | 40 | |

[a] Grove treated in May 1957; adult emergence occurred in June 1957. Each treatment was replicated twice. Organic matter content in this grove at the time of disking was low. Soil type: Coachella fine sand.

[b] Ten 1-square-yard emergence cages were set up in each plot. Results of all three insecticidal treatments were significantly different from the check at 0.01 level. Similarly, results of DDT treatments were significantly different from those of aldrin and toxaphene at the 0.01 level.

Table 5.—Emergence of *Hippelates* eye gnats from soil treated with Thiodan sprays in Tom Sakais young citrus grove, Indio, California.[a]

| Pounds Toxicant/ Acre | Average No. of Gnats/Cage and Reduction (%) | | | |
|---|---|---|---|---|
| | *H. collusor* | | *H. hermsi* | |
| | Gnats/ Cage | Reduction (%) | Gnats/ Cage | Reduction (%) |
| 2 | 160 | 3 | 8 | 43 |
| 4 | 154 | 7 | 11 | 20 |
| 6.2 | 101 | 39 | 7 | 50 |
| Check | 165 | — | 14 | — |

[a] Each treatment was replicated three times at random. Sprays were applied September 24, 1957, and emergence was recorded in October, 1957. Type of soil: Coachella fine sand. Ten 1-square-yard cages were set up in each plot. Differences were not significant.

or the aldrin treatments (table 4). Results with toxaphene and aldrin treatments were significantly different from those of the control. The long-term effectiveness of DDT and toxaphene, along with that of a few other chlorinated hydrocarbon insecticide sprays is currently under study.

Limited trials with Thiodan®(6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) sprays showed that this material had low biological activity against *Hippelates* gnats. In a test in a young citrus grove where this material was applied at various rates, control of both *H. collusor* and *H. hermsi* was relatively low at the highest practicable dosage of 3.2 pounds actual toxicant per acre (table 5). No significant difference appeared among the various Thiodan treatments and the check.

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# Field Testing of Insecticides for Control of the Nantucket Pine Moth, *Rhyacionia frustrana*, and the European Pine Shoot Moth, *R. buoliana*[1]

David E. Donley[2]

## Abstract

In field tests during 1957 and 1958 for control of the Nantucket pine moth, *Rhyacionia frustrana* (Comst.), and the European pine shoot moth, *R. buoliana* (Schiff.), it was found that emulsion formulations of Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (12 oz. actual), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate (12 oz. actual), malathion (2 lbs. actual), methyl parathion (1 lb. actual), and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) (12 oz. actual) gave control as good as or better than DDT (7 lbs. actual) when applied with a knapsack sprayer at a rate of 45 gallons of finished spray per acre. The effectiveness of control was based on a damage estimate obtained from counting all the infested and noninfested tips on treated and untreated check trees.

Among the terminal-feeding Lepidoptera of the genus *Rhyacionia* are found some of our most important forest and Christmas-tree-plantation insects (*R. frustrana*, *R. rigidana* (Fern.) and *R. buoliana*) that attack the pines planted in the Central States. Damage, especially to two of our more extensively used reforestation pines—red, *Pinus resinosa* Ait., and shortleaf, *P. echinata* Mill.—may range from distortion of leaders, thus causing the tree to be useless for ornamental or timber purposes, to complete stunting of the plants. Damage has been severe enough, as in the eastern counties of Ohio, to produce 4-foot red pines

[1] Accepted for publication November 18, 1959.

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Table 1.—Toxicants tested and effectiveness of control.[a]

| Toxicant | Pounds Toxicant per Acre[b] | Per Cent Tip Moth Control | | Per Cent Shoot Moth Control | |
|---|---|---|---|---|---|
| | | 1957 | 1958 | 1957 | 1958 |
| Aldrin emulsion | 3 | 30 | 26 | 45 | 39 |
| Chlordane emulsion | 1 | 16 | 11 | 25 | 22 |
| DDT emulsion | 7 | 89 | 88 | 91 | 91 |
| Delnav emulsion | ¾ | 93 | 96 | 93 | 95 |
| Dieldrin emulsion | 1 | 52 | 44 | 33 | 35 |
| Endrin emulsion | 1 | 83 | 78 | 79 | 77 |
| Guthion emulsion | ¾ | 94 | 93 | 95 | 95 |
| Heptachlor emulsion | 1 | 32 | 15 | 32 | 26 |
| Ronnel wettable powder | 1½ | 56 | 55 | 71 | 78 |
| Lethane emulsion | 2 | 37 | 16 | 45 | 42 |
| Malathion emulsion | 2 | 92 | 89 | 89 | 92 |
| Methyl parathion emulsion | 1 | 91 | 91 | 90 | 91 |
| Sevin® suspension | ½ | 45 | 36 | 60 | 67 |
| Strobane® emulsion | 2 | 18 | 12 | 36 | 34 |
| TEPP emulsion | ¾ | 35 | 36 | 56 | 66 |
| Toxaphene emulsion | 2 | 18 | 11 | 40 | 36 |
| Trithion emulsion | ¾ | 94 | 95 | 94 | 97 |
| Kepone emulsion | 2 | 29 | 38 | 42 | 48 |

[a] Based on no less than 48 replications per toxicant. The smallest number of 48 replications was that for TEPP, when adverse winds prevented treatment of some of the trees.
[b] Applied in 45 gallons of water.

in 14 years. Damage does appear to vary with site, but some shortleaf pines on good sites have been repeatedly infested over the past three seasons and have averaged only 2 inches of additional growth per year.

This paper reports the progress of the insecticide-screening program for control of terminal-feeding pests being carried out by the Central States Forest Experiment Station and the Ohio Division of Forestry. Specifically the report deals with tests of several insecticides on the Nantucket pine moth, *R. frustrana*, and the European pine shoot moth, *R. buoliana*, conducted during the summers of 1957 and 1958. The results given do not constitute recommendations for control.

Material and Methods.—The experimental design for all the field tests reported here was complete random of individual trees (replicates). The Nantucket pine moth tests were carried out on shortleaf pines 3 to 9 feet tall located on the Wayne-Hoosier National Forest near New Straitsville, Ohio. The European pine shoot moth tests were carried out using red pines 4 to 10 feet tall on the Maumee State Forest near Toledo, Ohio. The 1957 tests for each of the 2 insects were conducted using 14 individual trees selected at random from a block of 266 trees of the particular species concerned. The 1958 tests involved 60 individual trees per toxicant, selected at random from a block of 1,140 trees per plot. Each group of 60 trees was divided into 3 sets of 20 trees to facilitate application.

The present system of chemical control for this group of insects consists of completely drenching the tree with a toxicant in large volumes of water with hydraulic equipment or in large volumes of air with mist-blower sprayers. These methods are practically impossible for forest plantations in much of the Central States because of rough terrain. The materials tested in these experiments (table 1) were applied with a 3-gallon, knapsack-type sprayer using 45 gallons of water per acre. The desired dosage per acre was obtained by calibrating the sprayer and timing each individual treatment. Treatments were applied to control the early instars of the pest concerned and were timed, with the noted exceptions, to coincide with 50% adult emergence. The amount of toxicant used per acre was usually based on the manufacturer's suggestion, as no laboratory toxicity data were available. The dosage of 7 pounds of DDT per acre was used as a performance standard for mathematical comparison. Effectiveness of control was based on damage, using per cent of tips infested on treated vs. nontreated trees. Thus, we have a percentage control of damage, not of insects.

The data presented here are based on the use of a modification of Abbott's (1925) correction for the per cent control figures. They were analyzed, using analysis of variance, with individual observations being first transformed by the method of Curtiss (1943).

Chemical definitions of materials not bearing common names adopted by the Entomological Society of America appear at the end of this paper.

Field Test No. 1—Nantucket Pine Moth—1957.—During 1957, adults were observed for a period of 20 days in the study area. The counting of infested tips required 10 days of field observations, but since control estimates were based on damage and not insects, the error thus introduced was constant (table 1). Analysis of the data showed only Guthion® as significantly better than the DDT "standard." No significant differences could be detected between the DDT standard and the Delnav,® malathion, methyl parathion, and Trithion® effects.

Field Test No. 2—Nantucket Pine Moth—1958.—In this test each of the materials was applied to 60 shortleaf pines over a 3-day period (20 trees per toxicant per day). During this period no variation in the status of 50% adult emergence, used to time the application, could be detected. Adults in 1958 were observed throughout a 39-day period. Damage estimates required 15 days for completion (table 1).

Analysis of these data showed significant differences between the DDT standard and Delnav, Guthion, and Trithion. No significant differences could be detected between DDT and malathion and methyl parathion.

The controls for 1958 were generally poorer than those for 1957, probably because of the much longer oviposition period in 1958. Equal control for the 2 years would have required the toxicants to be persistent for nearly twice as long in 1958 as in 1957.

Field Test No. 3—European Pine Shoot Moth—1957.—Treatment of all replicates required 7 hours. The treatments were applied at 60% adult emergence owing to rain at the 50% emergence date.

Counts of damage required 3 days. Analysis of data from this test showed that only the Guthion controls were better than those obtained with the DDT standard. The Delnav, malathion, methyl parathion, and Trithion formulations gave controls not significantly different from those obtained with DDT.

Field Test No. 4—European Pine Shoot Moth—1958.—The treatments were divided (as with the 1958 tip moth test) into 3 sets of 20 trees. Thus, on each of 3 successive days, 360 were treated. The 3-day treatment period covered adult emergence from 50% to 60%.

Damage estimates required 9 days (table 1). Analysis of

the data showed significant differences between controls with the DDT standard and the Delnav, Guthion, and Trithion formulations. No significant differences could be detected between the DDT and malathion and methyl parathion treatments.

CONCLUSION.—From the results of these tests it would appear that emulsion formulations, applied with a knapsack sprayer at the rate of 45 gallons of finished spray per acre, timed to control the early larval instars of the European pine shoot moth and the Nantucket pine tip moth, and containing 12 ounces of Delnav, 12 ounces of Guthion, 2 pounds of malathion, 1 pound of methyl parathion, or 12 ounces of Trithion were as good or better than 7 pounds of DDT. Since all these tests have been experimental, and no information has been obtained on the effects of these treatments on associated insects or on wildlife, further larger-scale tests are needed before recommendations can be made.

Chemical definitions of materials not bearing adopted common names:

Delnav (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)

Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphoro-dithioate)

Kepone® or General Chemical 1189 (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one)

Lethane emulsion (53% by weight β-butoxy-β-thiocyanodiethyl ether plus 47% petroleum distillate)

Sevin (1-naphthyl-*N*-methyl carbamate)

Strobane (A mixture of chlorinated terpenes with about 66% chlorine)

Trithion (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)

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# Chlorinated Hydrocarbon Insecticides as Soil Treatments Against the Eye Gnat *Hippelates collusor* (Townsend) in the Laboratory[1,2]

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## ABSTRACT

A procedure for screening residual insecticides applied to soil in the laboratory has been perfected. An evaluation of some of the chlorinated hydrocarbon insecticides commonly used in controlling other soil-inhabiting insect pests revealed the fact that a few of these have appreciable biological activity against the eye gnat, *Hippelates collusor* (Townsend). These materials with a high degree of activity are Shell Compound SD-4402 (1,3,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan), DDT, and endrin. Shell Compound SD-4402 gave 98% reduction of the emerging gnats when applied at 2.5 pounds actual material per 6-inch acre (1 acre surface area 6 inches deep). DDT at 18 pounds actual material per 6-inch acre gave 80%, while endrin at 5 pounds actual material per 6-inch acre gave 95% reduction of the emerging eye gnats from the treated soil. Aldrin, dieldrin, heptachlor, lindane, Compound GC-1189 or Kepone® (1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1. $0^{2,6}$ .$0^{3,9}$ .$0^{5,8}$] decan-4-one), toxaphene, and chlordane yielded poor results when applied at moderate dosages (4 to 20 lbs. per 6-inch acre).

Granulated formulations of DDT, particularly the higher percentage formulations, were found to perform poorly as compared with spray applications. Upon aging for 3 months, the effectiveness of 20% and 35% granulated formulations on attapulgite granules increased somewhat but did not equal that of DDT sprays. Ten per cent granulated formulations manifested increased effectiveness on aging and proved to be as effective as sprays after 3 months' aging.

The biological activity of aldrin and dieldrin decreased when these materials were aged in soil for 3 months; that of DDT and endrin remained the same. The activity of toxaphene increased upon aging for 3 months, but the overall activity of this chemical against the pest was very low.

*Hippelates* eye gnats breed predominantly in cultivated light soils that contain some sort of organic matter and a moderate amount of moisture (Mulla 1958). One possible approach to instituting control measures against eye gnats might be to incorporate residual insecticides into the soil. Owing to numerous variables, the process of testing potential soil insecticides in the field without prior knowledge of their relative biological activity against gnats is tedious as well as expensive. It usually takes a great deal of time to obtain conclusive results from trials carried on in the field.

An initial laboratory screening program is imperative to avoid unnecessary field testing of those insecticides that have little or no biological activity against eye gnats. The desirability of such a screening procedure has long been recognized, but the program could not be put into actual operation because of the lack of laboratory populations of the eye gnats. A recent successful laboratory-rearing procedure of the most predominant species of eye gnats in the Southwest, *Hippelates collusor* (Townsend) (Mulla & Barnes 1957), has made it possible to undertake

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such a screening program under controlled conditions.

A soil insecticide for use in a program for control of eye gnats should:

1) have appreciable biological activity against the pest;

2) remain active for a long period of time (at least 1 year);

3) not accumulate to hazardous levels in the soil, so that the residues prove phytotoxic to plants;

4) have relatively low mammalian toxicity.

This paper covers only the first step, namely, evaluating the relative effectiveness of residual insecticides against eye gnats. It deals with procedures and results of tests in which most of the chlorinated hydrocarbon insecticides used as soil treatments for other soil-inhabiting insect pests have been screened against a common species of eye gnats. Vertical distribution of larvae and pupae of the eye gnat in the soil medium utilized in the experimental procedure of evaluating insecticides has also been studied. The extent of movement of larvae in the treated soil column could possibly influence the effectiveness of an insecticide against this insect.

METHODS AND MATERIALS.—*Soil Preparation.*—A medium-grade sand was obtained from virgin desert in the Coachella Valley, California. The sand was placed in a large, forced-draft oven (160° F.) until all moisture was driven out. The soil, when dried, was sifted through a 40-mesh sieve, so that coarser debris and plant residue could be removed.

One-half to two-gallon quantities of this soil (11.5 lbs./gallon) were used per treatment. The quantities of soil were measured by both volume and weight. Volume measurement permitted calculation of dosage per 1-acre surface area 6 inches deep and also made possible division of the large sample into subsamples that occupied the same amount of space each time. Weight measurement determined and regulated the degree of packing. Sprays were made by adding to water calculated amounts of emulsifiable concentrates of the insecticides involved to make 100 ml. of finished spray. The spray formulations were applied with a de Vilbiss atomizer at a rate of 100 gallons finished spray per 6-inch acre of the soil. This amount corresponded to approximately 2.32 ml. of the finished spray over an area of 36 square inches of 1 gallon of soil, giving good coverage to the treated surface. Granulated formulations were weighed on an analytical balance and spread over the surface of the soil lots.

As soon as the sprayed surface became dry, the spray as well as the granule-treated soil was mixed thoroughly with a spoon. Organic matter at the rate of 60 gm. alfalfa meal, 30 gm. brewer's yeast, and 30 gm. sifted whole wheat flour was added to 1 gallon of the treated soil. Thus the total amount was about 2.3% organic matter on a dry-weight basis. The mixture was then thoroughly spoon-mixed (roll mixing in a tumbler was not satisfactory) and placed in pint jars. One gallon of the soil, after addition of organic matter, partially filled (up to the neck) ten 1-pint, narrow-mouthed jars. The jars were set on a bench in a randomized block form. The temperature of the room was maintained at 88° to 90° F.

*Addition of Water and Eye-Gnat Eggs.*—Preliminary tests with various amounts of water indicated a total amount of 170 ml. of water for each pint jar to give satisfactory results. At first, 140 ml. of water was gradually added to each jar. The additional two 15-ml. quantities were added later when the surface of the soil had dried.

Within 1 to 3 days after addition of the water, 200 eye-gnat eggs from a laboratory colony were added to each jar, except as indicated. The laboratory colony was established in the fall of 1957 from wild populations collected in the Coachella Valley in southern California. After addition of the eggs, the jars were tightly covered with the ring and a 50-mesh disc of strainer cloth (coarser meshes permitted escape of the emerging eye gnats before they could be counted, and finer meshes hindered aeration of the mixture). The emerging gnats under $CO_2$ anesthesia were counted and removed at 2- to 4-day intervals after the beginning of emergence.

*Aging.*—Some of the chlorinated hydrocarbon insecticides evaluated in the laboratory were also tested for short-term longevity in the soil, as measured against eye gnats. One- to two-gallon quantities of soil were used per treatment. The soil was treated with the spray or granulated formulations and mixed in the manner described above. The treated soil was then stored in gallon jars, and 500 ml. of water was added to each jar to keep the soil damp. The jars were covered with a piece of fine organdy and set in a room maintained at a temperature of 88° to 90° F.

After 90 to 100 days, the treated soil from the jars was air-dried. Organic matter in the quantities previously described was added to each lot of soil. The mixture was then processed as described above.

*Larval and Pupal Washings.*—In order to determine the vertical distribution of the larval and pupal stages of *H. collusor*, soil medium in pint jars was sectioned and washed for recovery of these immature stages. The washing operation was initiated 3 days after addition of the eggs to the medium. First, each section of the soil column in the jars was removed and washed with 100 ml. of water. The supernatant was vacuum-filtered through a black cloth on a Büchner funnel. A few of the larvae and most of the pupae were recovered in this washing. The same portion of the soil was then washed with 150 ml. of 30% glycerine in water. This solution floated out almost all the remaining larvae and pupae. The supernatant liquid was filtered as described above, and the larvae and pupae were counted on the black cloth.

Glycerine solution has advantages over salt solutions for floating out eye-gnat larvae. In the former, the delicate larvae can remain for some time without being damaged or killed, and live larvae are much easier to detect and count than those that are dead or moribund.

RESULTS AND DISCUSSION.—*Larval and Pupal Distribution.*—The larvae of *H. collusor*, after hatching from the eggs, worked down into the soil and then, as the larvae reached maturity (table 1), they moved back up to the surface. Within 1 to 3 days after start of hatching, most of the larvae were located in the top 1¼-inch layer. However, as development progressed, the larval population density decreased in this portion of the medium with a corresponding increase in the lower layer. The greatest density of larvae in the bottom layer was reached between 7 and 9 days after addition of eggs to the medium. At this time, considerable decrease was evident in the number of larvae in the top and middle layers. This marked differ-

Table 1.—Vertical distribution of larval and pupal stages of *Hippelates collusor* during their developmental period in soil medium in pint jars.

| Days After Addition of Eggs | No. of Larvae and Pupae Recovered From Various Sections of the Soil Column[a] | | | | | |
|---|---|---|---|---|---|---|
| | Top (1¼ in.) | | Middle (1¼ in.) | | Bottom (1½ in.) | |
| | Larvae | Pupae | Larvae | Pupae | Larvae | Pupae |
| 3 | 134 | 0 | 39 | 0 | 1 | 0 |
| 5 | 95 | 0 | 144 | 0 | 1 | 0 |
| 7 | 60 | 39 | 147 | 0 | 5 | 0 |
| 9 | 11 | 91 | 54 | 0 | 84 | 0 |
| 11 | 10 | 187 | 1 | 8 | 12 | 0 |

[a] Two jars were sectioned on each date. Since the agreement between the two jars for each layer was very close, total recovery is reported here.

ence was accompanied by an increase in pupal population in the top layer. From the data presented in table 1, it is obvious that almost all the pupae were confined to the top layer.

This information indicates that larvae of *H. collusor* penetrated the whole depth of the medium and thus came in contact with the toxicants that were added to it in the experiments described below. However, it should be pointed out that the total number of larvae encountered in the bottom of the medium during the total period of development was smaller than the total number found in the other layers. As a result of this heterogeneous distribution of larvae, the total number of days spent in the lower portion, times the number of larvae, would be much smaller in contrast to the other layers. This suggests that the toxicant dosages used here were slightly larger for a given kill than they were in situations where the larvae penetrated the medium uniformly.

*Insecticidal Treatments.*—In order to get an idea of the range of activity of some materials, preliminary tests were made with a few dosages of the insecticides before undertaking full-scale experiments. Some of the insecticides were tested first at lower or practical dosages. If the activity within this range was low, further tests were carried out, utilizing much higher rates.

The data obtained in some cases were analyzed statistically. Analysis of variance was completed by transforming the original data into angles. The results of these analyses are shown in footnotes in the various tables.

*Initial Effectiveness.*—DDT in the form of sprays within the range of practical dosages had good initial activity (table 2). Storage for 3 months (at 18 lb./6-inch acre) increased its effectiveness slightly over that of a freshly treated sample (see table 12). The activity of DDT increased linearly with an increase in dosage. Analysis of the data showed a nonlinear relationship between the dosage and the effect below the 6 pounds per 6-inch acre dosage. This indicates that somewhere below this dosage level there is no corresponding increase in effect as the dosage is increased.

Toxaphene sprays caused some reduction in the number of emerging eye gnats. There was a significant increase in kill as related to an increase in dosage. The kill at practical dosages (20 to 24 lb./6-inch acre), however, was very

Table 2.—Initial effectiveness of DDT sprays against *Hippelates collusor*.[a]

| Pounds Actual Material/6-Inch Acre[b] | Average No. Gnats/Jar[c] | Reduction (%) |
|---|---|---|
| 6 | 81.2 | 19.8 |
| 12 | 51.4 | 49.5 |
| 18 | 19.7 | 80.6 |
| Check | 101.3 | — |

[a] Each treatment replicated 10 times. DDT emulsifiable concentrate (2 lb./gallon) was used to prepare the finished spray.

[b] The dosage in this and in the following tables means that the indicated amount of the actual toxicant was applied to 1 acre of soil 6 inches deep.

[c] Among the various dosages of DDT, there is a linear relationship at the 0.001 level and a quadratic relationship at the 0.05 level, below the 6 lb./6-inch acre dosage.

low (table 3). It is possible that the activity of this material might increase on aging (see table 11), but the minimum dosage at which appreciable activity was manifested was relatively very high.

The addition of toxaphene (8 lb./6-inch acre) to various dosages of DDT resulted in greater reductions of emerging gnats at the lower two dosages of DDT than the corresponding additive reduction for the two materials used separately (table 4). At the highest dosage of DDT (18 lb./6-inch acre), the synergistic effect of toxaphene, however, was negligible. Since toxaphene in combination with DDT did not manifest a great deal of synergistic action, further trials were not undertaken. Moreover, the addition to soil of a residual insecticide such as toxaphene with

Table 3.—Reduction in emergence of *Hippelates collusor* with various dosages of toxaphene applied as sprays to the soil.[a]

| Pounds Actual/6-Inch Acre | Average No. Gnats/Jar[b] | Reduction (%) |
|---|---|---|
| 8 | 85 | 15 |
| 16 | 72 | 28 |
| 24 | 67 | 33 |
| 32 | 44 | 56 |
| Check | 100 | — |

[a] Toxaphene emulsifiable concentrate 8 lb./gallon was used to prepare the dilute spray.

[b] Each treatment replicated 10 times. There is a linear relationship among these dosages at the 0.001 level. The check is significantly higher than the other treatments at the 0.001 level.

Table 4.—Emergence of *Hippelates collusor* from soil treated with a spray prepared from emulsifiable concentrates of DDT and toxaphene.[a]

| Insecticide and Formulation | Pounds Actual Material/6-Inch Acre | Average No. Gnats/Jar | Reduction (%) | Additive Reduction (%)[b] |
|---|---|---|---|---|
| DDT, 2+Toxaphene, 8[d] | 6+8 | 41.2[c] | 56 | 36 |
| DDT, 2+Toxaphene, 8[d] | 12+8 | 18.7 | 80 | 66 |
| DDT, 2+Toxaphene, 8[d] | 18+8 | 7.8 | 92 | 90 |
| Check | — | 93.5 | — | — |

[a] Each treatment replicated 10 times.

[b] Percentage reduction by the two dosages of each insecticide when run separately.

[c] Within the three dosages there is a linear relationship at the 0.001 level. The check is significantly higher than the others at the 0.001 level.

Table 5.—Emergence of *Hippelates collusor* from soil treated with aldrin and heptachlor sprays.[a]

| Insecticide and Formulation | Pounds Actual Material/6-Inch Acre | Average No. Gnats/Jar | Reduction (%) |
|---|---|---|---|
| | *Experiment A*[b] | | |
| Aldrin EC 2 | 2.0 | 124.4 | 8.0 |
| | 4.0 | 121.6 | 9.7 |
| | 6.0 | 70.6 | 47.7 |
| | 8.0 | 55.8 | 58.7 |
| Heptachlor EC 2 | 2.0 | 123.6 | 8.0 |
| | 4.0 | 124.8 | 7.5 |
| | 6.0 | 122.8 | 9.0 |
| | 8.0 | 124.0 | 8.0 |
| Check | — | 135.0 | — |
| | *Experiment B* | | |
| Aldrin EC 2 | 10 | 32 | 72 |
| | 20 | 5 | 96 |
| | 30 | — | 100 |
| | 40 | — | 100 |
| Heptachlor EC 2 | 10 | 102 | 12 |
| | 20 | 111 | 3 |
| | 40 | 70 | 39 |
| | 60 | 48 | 58 |
| Check | — | 114 | — |

[a] Each treatment replicated 5 times.

[b] In Experiment A, the data were analyzed statistically and eye gnat emergence in aldrin treatments at the two highest dosages was found to be significantly lower than that in the check or in the heptachlor treatments. There is also a linear relationship among the various aldrin dosages at the 0.001 level.

a low level of activity would increase accumulation hazards.

Aldrin in economical dosages (2 lb./6-inch acre) showed very little biological activity. As the dosages were increased, this material manifested a high degree of initial activity (table 5). Heptachlor, on the other hand, proved to have low activity at moderate dosage levels. Its activity increased slightly with increase in dosage (table 5).

Lindane and chlordane manifested low biological activity, though the former exhibited slightly greater activity than the latter. At the lower rates, these materials showed very little activity (table 6). A greater reduction was obtained with lindane than with chlordane by doubling and tripling the lowest dosage.

Table 6.—Reduction in emergence of *Hippelates collusor* from soil treated with various dosages of chlordane and lindane.[a]

| Treatment | Pounds Actual Material per 6-Inch Acre | Average No. Gnats/Jar | Reduction (%) |
|---|---|---|---|
| Chlordane | 16 | 123 | 10 |
| | 32 | 100 | 27 |
| | 48 | 89 | 35 |
| Lindane | 10 | 120 | 11 |
| | 20 | 69 | 50 |
| | 30 | 33 | 76 |
| Check | — | 136 | — |

[a] Each treatment replicated 5 times.

Table 7.—Control of *Hippelates collusor* with various dosages of Compound GC-1189 applied as sprays.[a]

| Pounds Actual Material/6-Inch Acre | Gnat Emergence | | Reduction (%) |
|---|---|---|---|
| | Range | Average No./Jar | |
| 10 | 92–137 | 118 | 6 |
| 20 | 67–116 | 86 | 31 |
| 30 | 5– 77 | 50 | 60 |
| Check | 83–139 | 125 | — |

[a] Emulsifiable concentrate containing 2 lb. active material/gallon was used to prepare the sprays. Each treatment was replicated 5 times.

Compound GC-1189 or Kepone® (1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1. $0^{2,6}$ .$0^{3,9}$. $0^{5,8}$] decan-4-one) did not prove very effective even when applied at 30 pounds actual toxicant per 6-inch acre (table 7).

Shell Compound SD-4402 (1,3,4,5,6,7,8,8,-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) was the most effective of all the chlorinated hydrocarbons tested. It manifested high biological activity within the range of 2 to 3 pounds actual toxicant/6-inch acre (table 8). Studies on the long-term effectiveness of this material are under way.

The effectiveness of dieldrin against *H. collusor* was very low at the practical dosage level (2 to 4 lb. actual material per/6-inch acre). This material did not show much activity until it was applied at the rate of 6 pounds per acre (table 9). Appreciable control was obtained only with dosages of 20 or 30 pounds per acre. Endrin, on the other hand, yielded promising results when used at lower dosages (see table 9), and a high degree of control when used at dosages of 3 to 5 pounds per acre.

*Effectiveness on Aging.*—Soil treated with some of the materials was stored in the laboratory and then investigated for short-term effectiveness. The treatment dosages for the given materials were those which lie at the upper end of the range of practical dosages. For some materials, this dosage yielded a high degree of control; for others, control was low to moderate.

Aldrin and dieldrin at the respective dosages of 8 and 6 pounds per acre yielded significantly lower control when aged for 3 months than they did initially (table 10). The loss of activity on aging was much higher for aldrin than for dieldrin. In the light of recent information and the

Table 8.—Effectiveness of SD-4402 against *Hippelates collusor* when applied as spray to soil in the laboratory.[a]

| Pounds Actual/6-Inch Acre | Average No. Gnats/Jar | Reduction (%) |
|---|---|---|
| 0.75 | 72.0 | 45.4 |
| 1.00 | 43.2 | 67.4 |
| 1.25 | 20.8 | 84.3 |
| 2.50 | 1.6 | 98.8 |
| 3.75 | 0 | 100 |
| 5.00 | 0 | 100 |
| 6.25 | 0.2 | 99.8 |
| 7.50 | 0 | 100 |
| Check | 132.0 | — |

[a] Each treatment replicated 5 times. SD-4402 emulsifiable concentrate containing 1.25 pounds actual material per gallon was used to prepare the sprays.

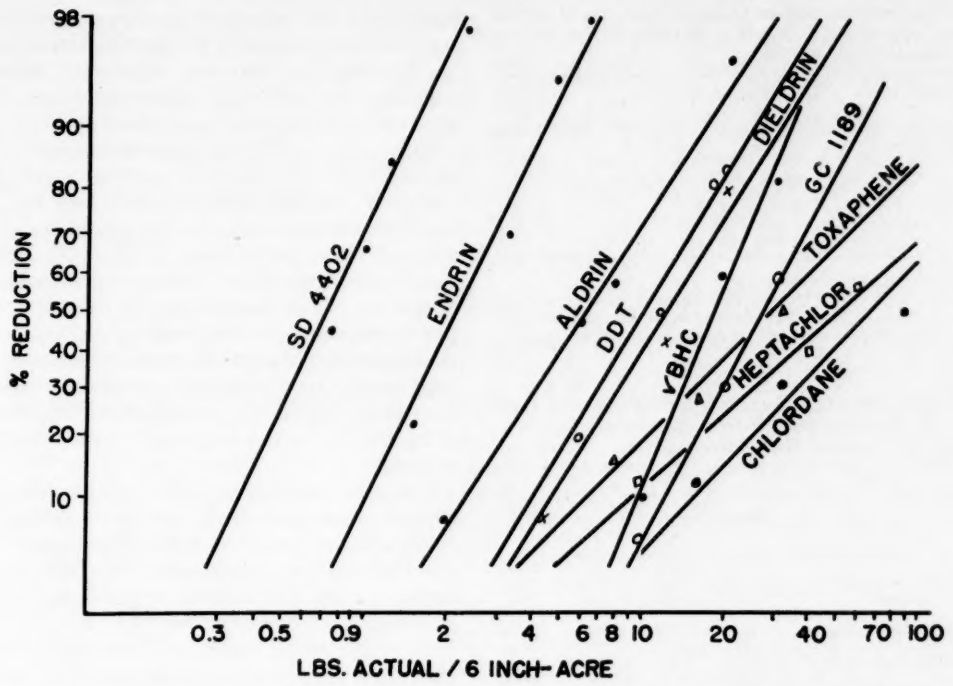


FIG. 1.—Dosage-mortality lines of the initial activity of various chlorinated hydrocarbon insecticides applied as sprays to soil in the laboratory for use against *Hippelates collusor* (Townsend).

data presented here, such a loss in initial activity of aldrin is rather to be expected. Recent evidence supporting the conversion of aldrin to its epoxide (dieldrin) in soil (Bollen *et al.* 1958, Gannon & Bigger 1958) satisfactorily explains this phenomenon. Incomplete conversion of aldrin to dieldrin results in the presence of lower dosages of both these materials, at which very low activity against the gnat is manifested (see tables 5 and 9). Even with a quantitative conversion of aldrin to dieldrin, the degree of reduction would be decreased as a result of the much lower biological activity of dieldrin (see fig. 1).

The conversion of aldrin to a biologically less active compound, dieldrin, is undesirable in a program for control of *H. collusor*. However, for the control of other insect pests, this conversion to dieldrin, a material which proved to be more toxic and residual than aldrin, was considered advantageous (Gannon & Bigger 1958). The conversion of heptachlor to its epoxide in the soil has also been demonstrated (Gannon & Bigger 1958). Thus the lower initial activity of heptachlor, as well as the short-term activity, may also be due to its conversion to its epoxide, which probably has a very low degree of activity against the gnat.

**Table 9.—Emergence of *Hippelates collusor* from soil treated with various dosages of dieldrin and endrin applied as sprays in the laboratory.[a]**

| Insecticide and Formulation | Pounds Actual Material/6-Inch Acre | Average No. Gnats/Jar | Reduction (%) |
|---|---|---|---|
| | *Experiment A*[b] | | |
| Dieldrin EC 1.5 | 1.5 | 102.4 | 0.0 |
| | 3.0 | 86.4 | 7.5 |
| | 4.5 | 85.6 | 8.0 |
| | 6.0 | 66.4 | 29.0 |
| Endrin EC 1.6 | 1.6 | 72.8 | 22.6 |
| | 3.2 | 22.6 | 75.7 |
| | 4.8 | 5.2 | 94.4 |
| | 6.4 | 1.2 | 98.7 |
| Check | — | 93.0 | — |
| | *Experiment B* | | |
| Dieldrin EC 1.5 | 12 | 83 | 39 |
| | 20 | 34 | 75 |
| | 30 | 2 | 99 |
| Check | — | 136 | — |

[a] Each treatment replicated 5 times.
[b] There is a linear relationship between the various dosages of dieldrin and endrin at the 0.001 level.

**Table 10.—Effect of storage on the biological activity of aldrin and dieldrin sprays used as soil treatments against *Hippelates collusor*.[a]**

| Insecticide and Formulation | Pounds Actual Material/6-Inch Acre | Emergence of Gnats and Reduction (%) | | | |
|---|---|---|---|---|---|
| | | Initial | | After 3 Months' Storage | |
| | | Average No. Gnats/Jar | Reduction (%) | Average No. Gnats/Jar | Reduction (%) |
| Aldrin EC 2 | 8 | 64.7[b] | 50.0 | 111.9[b] | 11.0 |
| Dieldrin EC 1.5 | 6 | 90.3[c] | 29.0 | 111.4[c] | 11.5 |
| Check | — | 126.3[d] | — | | |

[a] Each treatment replicated 10 times.
[b] Significantly different from each other at the 0.001 level.
[c] Significantly different from each other at the 0.01 level.
[d] Check significantly different from the other treatments at the 0.001 level.

**Table 11.—Effect of storage on biological activity of endrin and toxaphene sprays used as soil treatments in the laboratory against *Hippelates collusor*.[a]**

| Insecticide and Formulation | Pounds Actual/6-Inch Acre | Average No. Gnats/Jar and Reduction (%)[b] | | | |
|---|---|---|---|---|---|
| | | Initial | | After 98 Days' Storage | |
| | | Gnats/Jar | Reduction (%) | Gnats/Jar | Reduction (%) |
| Endrin EC 1.6 | 6.4 | 7.8 | 93.5 | 6.9 | 94.3 |
| Toxaphene EC 8 | 24.0 | 95.2 | 20.0 | 69.3 | 42.0 |
| Check | | 119.4 | | | |

[a] Each treatment replicated 10 times.
[b] No significant difference exists between fresh and stored endrin treatments. Fresh toxophene treatment is significantly different at the 0.01 level from toxaphene treatment that was stored. Check is significantly different from the other treatments at the 0.001 level.

**Table 12.—Effectiveness of various formulations of DDT (at 20 lb. actual material per 6-inch acre) initially and after 3 months' storage, against *Hippelates collusor*.**

| DDT Concentration and Formulation | Initial Effectiveness | | Effectiveness After 3 Months' Storage | |
|---|---|---|---|---|
| | Average No. Gnats/Jar | Reduction (%) | Average No. Gnats/Jar | Reduction (%) |
| 10% on 30/40 RVM-A[a] attapulgite | 59.0[b] | 44.5 | 20[c] | 81.0 |
| 20% on 30/60 RVM-A[a] attapulgite | 101.0 | 6.0 | 72.2[d] | 30.0 |
| 35% on 30/60 RVM-A[a] attapulgite | 106.0 | — | 64.7[d] | 37.0 |
| Spray EC 2[e] | 18.6[b] | 82.5 | 16.5[c] | 84.0 |
| Check[e] | 106.7 | — | 102.7 | — |

[a] Replicated 20 times.
[b] Significantly different from each other and the rest of the treatments at the 0.01 level.
[c] Significantly different from the rest of the treatments at the 0.01 level.
[d] Significantly different from the check at the 0.01 level.
[e] Replicated 10 times.

Toxaphene-treated soil at 24 pounds per acre resulted in a significantly higher degree of control after it had been stored for 98 days than it did initially (table 11). The effectiveness of endrin at 6.4 pounds per acre after storage was about the same as its initial activity (table 11). This fact suggests that under the conditions of the experiment, there is no breakdown of this material into less toxic products.

The comparison of a DDT spray and various DDT granulated formulations (on attapulgite granules) at 20 pounds active ingredient per 6-inch acre indicated that the initial effectiveness of granules decreased as the percentage concentration of the active material in the granulated formulation increased (table 12). The granulated formulations proved to be significantly less active initially than the spray formulation. After 3 months' storage, the release from the 10% granulated formulation was complete, and the results were equal to those of the spray treatment. Granulated formulations of 20% and 35%, however, did not release enough toxicant after this period and performed poorly in comparison with spray and 10% granulated formulations. This poor performance and slow release of the active material from the inert particles of the higher concentration may be explained in terms of (a) the low vapor pressure of the insecticide, (b) the low total surface area from which insecticide vapors may diffuse, and (c) the fewer granular particles per unit volume of treated soil, which will result in poor distribution and coverage.

From the results of this series of experiments (fig. 1), it is obvious that field evaluation and long-term trials with chlorinated hydrocarbon insecticides against *H. collusor* should be confined to those materials that yielded promising results against this pest in the laboratory. These materials are Compound SD-4402, endrin, aldrin, and DDT. Further studies on long-term effectiveness, accumulation pattern in the soil, and phytotoxicity of these materials are needed before any one of these materials is used in a large-scale control program against eye gnats. Mammalian toxicity and the type of formulation will also play a role in the final selection of such a material.

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# Effect of Ronnel[1] upon the Adult Rice Weevil, *Sitophilus oryza*[2]

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### Abstract

Laboratory studies using Hard Red Winter wheat treated at 1.85 and 4.00 p.p.m. with ronnel, sampled as Korlan 24 E, produced mortalities of 50% and 95%, respectively, immediately following a 14-day exposure. A dosage of 1.00 p.p.m. produced a mortality of only 7% but reduced reproduction approximately 94%. Odor, feel or appearance of the wheat was unaffected at 5.0 p.p.m.; however, 10.0 and 25.0 p.p.m. produced odors.

Many types of toxicants and/or repellents have been tested experimentally as potential grain protectants. Combinations of pyrethrins and piperonyl butoxide are used extensively as grain protectants (Dove 1947, Wilbur 1952, and others). Malathion dust or spray formulations are also used to protect wheat (Lindgren *et al.* 1954, Watters 1959). Hornstein *et al.* (1958) reported 100% mor-

[1] Coined name for *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate. Also known as Dow ET-57 sampled as Korlan 24E. Supplied by the Dow Chemical Company.

[2] Contribution No. 752, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Investigations conducted under the direction of D. A. Wilbur. Accepted for publication November 19, 1959.

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tality of confused flour beetles, *Tribolium confusum* Duv., when exposed to vapors of ronnel at 24° to 33° C. The insecticide is also effective against other insects. A complete knockdown of house flies, *Musca domestica* L., was obtained in 111 minutes with ronnel (Ihndris & Sullivan 1958). Ronnel was superior to malathion in control of house fly larvae in chicken droppings (Knapp *et al.* 1958). Many accounts of its effectiveness for control of cattle grub (*Hypoderma* spp.) appear in the literature.

This laboratory study was conducted during the spring of 1959 to determine the effectiveness of ronnel as a wheat protectant against the adult rice weevil, particularly because of its effectiveness against insects and its relatively low mammalian toxicity.

MATERIALS AND METHODS.—No. 1, Hard Red Winter wheat harvested in 1956 was cleaned to reduce dockage to less than 1%. Fifty-pound lots were stored at 0° F. 7 days to kill any existing infesting insects. The temperature was increased to approximately 80° F. and moisture content determined with a Model G Steinlite moisture tester. Distilled water was added to temper to approximately 13% in 55-gallon barrels. Each barrel was rolled 30 minutes on each of 4 consecutive days before the desired moisture equilibrium was obtained. When the moisture content tested 13.0+0.3% at the termination of the tempering process, the wheat was used immediately for experimentation.

The tempered wheat was divided into lots of 1,797 grams and each lot was placed in a separate 1-gallon glass jar. Ronnel was diluted with distilled water to apply specific concentrations at the rate of 1.00 ml. per 1,797 grams of wheat (equivalent to 4.0 gallons per 1,000 bushels). The dilution was applied to the interior walls of the glass jar above the level of wheat using a 1.0 ml. volumetric glass pipette. Each jar was immediately hand shaken 2 minutes to spread the liquid around the wheat kernels and then rotated an additional 20 minutes on a mechanical tumbler. The jar lids were removed to expose the treated wheat for 24 hours to air under room conditions. Four replicates of 400 grams each were removed from the 1,797-gram wheat samples. Each replicate was placed in a 1-quart wide mouth Mason jar. Excess wheat was discarded.

All weevils were reared and test weevils were stored at 80+2° F. and 70+5% relative humidity. Cultures were started every 3 days to provide a constant supply of insects.

*Toxicity Tests.*—Fifty adult weevils at 14+1.5 days old were released into each 400-gram replicate. Self-sealing Mason jar lids were inverted to allow some air passage but still to retain the weevils.

Following the desired exposure period, the weevils were separated from the wheat with a #10 wire screen on a mechanical shaker 30 seconds and observed for mortality. Insects were considered alive if capable of any movement when stimulated by vibration, light, or mild heat.

*Reproduction.*—After the adult weevils were removed, the treated wheat was returned to the 1-quart jars and stored 35 days. The samples were then shaken on a #10 wire screen and the number of progeny recorded.

*Length of Exposure Test.*—Ten lots of wheat, 1,797 grams each, were treated with ronnel at a dosage of 2.5 p.p.m. and thoroughly mixed following a 24-hour aeration period. Thirty samples of 400 grams each were placed in 1-quart glass jars and the excess wheat discarded. Fifty adult weevils were introduced into each of the replicated samples and then the samples were stored. Adults were screened and recorded from 3 replicates every 3 days for a total of 10 sampling periods. These replicates were then discarded.

**Table 1.—Percentage mortality of adult rice weevils exposed 14 days to wheat[a] treated with various dilutions of ronnel at the rate of 4.0 gallons of total spray per 1,000 bushels.**

| | Percentage Mortality in Replicates | | | | |
|---|---|---|---|---|---|
| Dosage (p.p.m.)[b] | 1 | 2 | 3 | 4 | Average |
| 0.0 | 0 | 2 | 0 | 2 | 1 |
| 1.0 | 10 | 8 | 8 | 2 | 7 |
| 1.4 | 24 | 35 | 12 | 20 | 23 |
| 1.8 | 49 | 24 | 45 | 52 | 43 |
| 2.2 | 72 | 74 | 71 | 65 | 71 |
| 2.6 | 72 | 86 | 80 | 82 | 80 |
| 3.0 | 83 | 86 | 86 | 84 | 85 |
| 3.4 | 88 | 92 | 90 | 96 | 92 |
| 3.8 | 94 | 93 | 90 | 94 | 93 |
| 4.2 | 96 | 98 | 96 | 92 | 96 |
| 4.6 | 93 | 98 | 96 | 100 | 97 |
| 5.0 | 100 | 98 | 96 | 98 | 98 |

[a] No. 1, 1956, Hard Red Winter wheat at 13.0±0.3% moisture, less than 1.0% dockage and 80±3° F.
[b] Parts per million of active ingredient to wheat by weight.

*Rate of Toxicant Deterioration.*—Adults were exposed 14 days to wheat treated approximately 2 months previously at the relatively high concentrations of 10, 20, 30, and 40 p.p.m. Adults were then separated with a #10 wire screen and observed for mortality.

*Effect on Wheat.*—Wheat treated with ronnel at dosages of 0.0, 5.0, 10.0, and 25.0 p.p.m. was graded by feel, appearance, and odor after a 3-month aeration period at room conditions.

RESULTS AND DISCUSSION.—*Toxicity Tests.*—Table 1 shows the percentage mortality of weevils exposed 14 days to wheat treated at various dosage levels. At 2.6 p.p.m., 80% mortality was obtained but 5.0 p.p.m. failed to produce 100% mortality except in one replicate. The dosage mortality curve fitted by eye shown in figure 1 indicates $LC_{50}$ and $LC_{95}$ values of 1.85 and 4.0 p.p.m., respectively.

*Reproduction.*—Progeny counts 35 days after 14-days' exposure revealed averages of 12, 8, and 5 adults per replicate from wheat treated at 20.0, 30.0, and 40.0 p.p.m., respectively (table 2); however, even these weevils were dead at the time of this inspection. Both dead and living adults in a ratio of approximately 3 to 1 were observed in wheat dosed at 1.0 or 10.0 p.p.m. At the relatively low dosage of 1.0 p.p.m. the average number of adults, living and dead, was only 5.9% of the adults which emerged from the untreated check samples. A dosage of 0.1 p.p.m. had little effect on the reproductive potential of the weevils.

*Length of Exposure Tests.*—The percentage mortality of weevils exposed for various periods to wheat treated at a dosage of 2.5 p.p.m. is shown in table 3. Nine days' exposure produced 80% mortality but increasing the exposure to 27 days increased mortality only to 93%.

*Rate of Toxicant Deterioration.*—A dosage of 20 p.p.m.

Table 2.—Rice weevil progeny 35 days after a 14-day exposure period in wheat[a] treated with various dilutions of ronnel at the rate of 4.0 gallons of total spray per 1,000 bushels.

| Dosage (p.p.m.)[b] | No. of Rice Weevil Progeny in Replicates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | Average | |
| | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead |
| 0.0 | 896 | 0 | 1,164 | 0 | 435 | 0 | 1,026 | 0 | 880 | 0 |
| 0.1 | 952 | 0 | 683 | 0 | 715 | 0 | 803 | 0 | 788 | 0 |
| 1.0 | 10 | 37 | 18 | 36 | 11 | 43 | 8 | 42 | 12 | 40 |
| 10.0 | 14 | 13 | 11 | 45 | 9 | 50 | 14 | 31 | 12 | 35 |
| 20.0 | 0 | 6 | 0 | 10 | 0 | 14 | 0 | 17 | 0 | 12 |
| 30.0 | 0 | 5 | 0 | 5 | 0 | 14 | 0 | 0 | 0 | 8 |
| 40.0 | 0 | 3 | 0 | 0 | 0 | 8 | 0 | 10 | 0 | 5 |

[a] No. 1, 1956, Hard Red Winter wheat at 13.0±0.3% moisture, less than 1.0% dockage and 80±3° F.
[b] Parts per million of active ingredient to wheat by weight.

or greater produced 100% mortality after 14 days' exposure to wheat that had been treated approximately 2 months previously (table 4).

*Effect on Wheat.*—Appearance or feel was not affected at any dosage level tested. No odor was detected at 5.0 p.p.m.; however, 10.0 p.p.m. produced a questionable odor and 25.0 p.p.m. retained an objectionable odor. These effects were observed by the Board of Grain Supervisors of the United States Department of Agriculture, following a 3-month aeration period after wheat treatment.

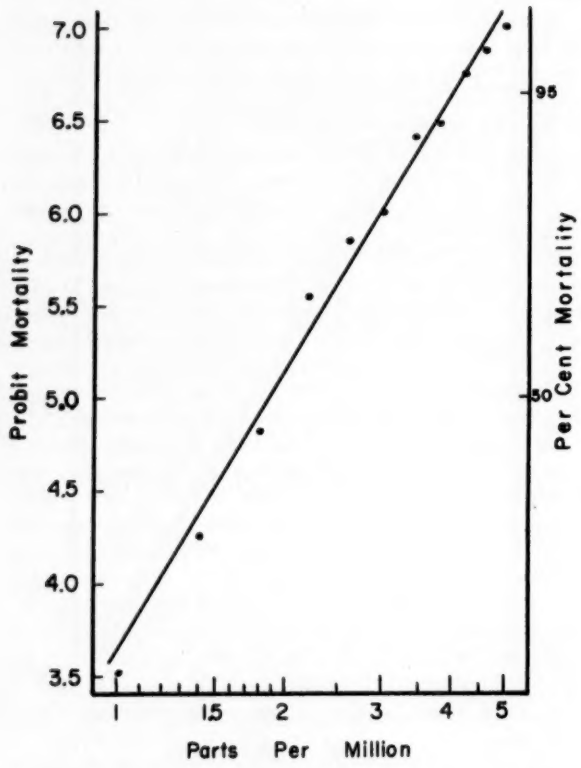


FIG. 1.—Dosage mortality curve for adult rice weevils (*Sitophilus oryza*) exposed 14 days in wheat treated with ronnel at the rate of 4.0 gallons per 1,000 bushels. (No. 1, 1956, Hard Red Winter wheat at 13.0±0.3% moisture, less than 1.0% dockage, and 80±3° F.)

Table 3.—Percentage mortality of adult rice weevils exposed for various periods to wheat[a] treated with 2.5 p.p.m.[b] of ronnel at the rate of 4.0 gallons of total spray per 1000 bushels.

| Exposure Period (Days) | Percentage Mortality in Replicates | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Average |
| 3 | 8 | 4 | 10 | 7 |
| 6 | 46 | 56 | 56 | 53 |
| 9 | 84 | 70 | 86 | 80 |
| 12 | 82 | 84 | 72 | 79 |
| 15 | 85 | 84 | 90 | 86 |
| 18 | 82 | 84 | 88 | 85 |
| 21 | 86 | 84 | 94 | 88 |
| 24 | 90 | 92 | 88 | 90 |
| 27 | 98 | 88 | 92 | 93 |
| 30 | 98 | 90 | 90 | 93 |

[a] No. 1, 1956, Hard Red Winter wheat at 13.0±0.3% moisture, less than 1.0% dockage and 80±3° F.
[b] Parts per million of active ingredient to wheat by weight.

Table 4.—Percentage mortality of adult rice weevils exposed 14 days to wheat[a] treated with various dilutions of ronnel at the rate of 4.0 gallons of total spray per 1,000 bushels, approximately 2 months prior to exposing the insects.

| Dosage (p.p.m.)[b] | Percentage Mortality in Replicates | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Average |
| 40 | 100 | 100 | 100 | 100 | 100 |
| 30 | 100 | 100 | 100 | 100 | 100 |
| 20 | 100 | 100 | 100 | 100 | 100 |
| 10 | 66 | 87 | 65 | 72 | 73 |

[a] No. 1, 1956, Hard Red Winter wheat at 13.0±0.3% moisture, less than 1.0% dockage and 80±3° F.
[b] Parts per million of active ingredient to wheat by weight.

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# Development of Granary Weevils and Storage Fungi in Columns of Wheat—II[1]

C. M. CHRISTENSEN and A. C. HODSON[2]

ABSTRACT

Seed-grade wheat with a moisture content of 13% to 14% was stored in "Transite" cylinders 8 inches in diameter and 40 inches long, each of which held 23 kg. of grain. Two hundred adults of *Sitophilus granarius* (L.) were added to the grain in each of two cylinders and allowed to wander freely. Two hundred adult weevils were added to 2.4 kg. of grain in each of two brass cloth cages, one of which was placed at the bottom of each of another pair of cylinders, which were then filled with grain. Another pair of cylinders filled with grain served as controls. The cylinders were kept at 25° C. and 65% to 70% relative humidity. Samples of wheat were withdrawn through side ports, with minimum disturbance to the bulk, after 2, 4, and 6 months, and tested for moisture content, fungus population, germination, insect damage, and number of insects. Where insects were not confined, the moisture content increased to 20% in the upper portion of the grain; throughout the bulk, moisture content increased several per cent and storage fungi increased greatly. The greatest increase in temperature was approximately 6° C. (10.8° F.). Where insects were confined at the bottom of the column of grain, moisture content increased as much as 3% in the grain several inches above the infested portion and approximately 0.5% in the grain 1 to 2 feet above the infested portion. The grain in the controls remained essentially unchanged. Fumigation with a commercial formulation killed all the insects but had no effect on the population of storage fungi which continued to increase after fumigation.

Agrawal *et al.* (1957, 1958) found a close relationship between the granary weevil, *Sitophilus granarius* (L.), and certain fungi that cause damage in stored grains, especially *Aspergillus restrictus* Smith. In columns of wheat, moisture content and storage fungi increased greatly where the weevils congregated; when the weevils were confined at the bottom of the column of grain, moisture content and storage fungi increased in the grain immediately above, and proportionally at least equal in volume to, that occupied by the insects. In those tests the columns of grain were kept in a room at a low relative humidity, resulting in some loss of moisture from the grain in the control columns and presumably also from the grain in which the insects were present. The aim of the present tests was to store somewhat larger volumes of grain than those previously used, at a relative humidity approximately in equilibrium with the grain at the beginning of the tests, and in columns from which only minor evaporation could occur.

MATERIALS AND METHODS.—The columns or cylinders were sections of "Transite" pipe which was 40 inches long and 8 inches in internal diameter and had walls $\frac{3}{8}$ inch thick. Holes 1 inch in diameter were bored at intervals of 5 inches in a row from near the bottom to near the top on one side of the cylinder, to allow removal of samples by means of a small probe. Except when samples were being taken, the holes were closed with No. 5 rubber stoppers. The inner wall of the cylinder was coated with hot paraffin to make it relatively impervious to water, and the ends of each cylinder were covered with boards approximately 12 inches square, also paraffin-coated on the inner side. Two metal rods with screw threads on the upper end were passed through holes in the wood end covers, and wing nuts were screwed down so that the covers fitted tightly to the cylinder ends. Calking compound was pressed into the joint of cylinder and wood cover to complete the seal. Figure 1 shows the cylinder. A hole $\frac{3}{16}$ inch in diameter was bored through the rubber stopper in the bottom port or hole of each cylinder, and the outer end of this hole was covered with a 40-mesh screen. A hole 2 inches in diameter was bored through the top wood cover, and similarly covered with screen. It was thought that these openings would provide sufficient ventilation to prevent accumulation of carbon dioxide without allowing much evaporation, and apparently they served the purpose very well.

The grain used was a lot of seed-grade, hard, red spring wheat grown on University plots in 1958 and stored at a moisture content of approximately 10% from harvest until it was used. Approximately 23 kg. of wheat were weighed for each cylinder and placed in a metal tub. Distilled water was added to bring the moisture content to 13% to 14%. The grain was mixed thoroughly, covered and allowed to stand for several hours, mixed again, and poured into the cylinder. After the grain had been in the cylinder for several days, samples were removed from ports at the bottom, middle, and top and tested for moisture content. If the moisture content of the three samples ranged more than ± 0.2% from the average, the grain was poured out, remixed, and returned to the cylinder;

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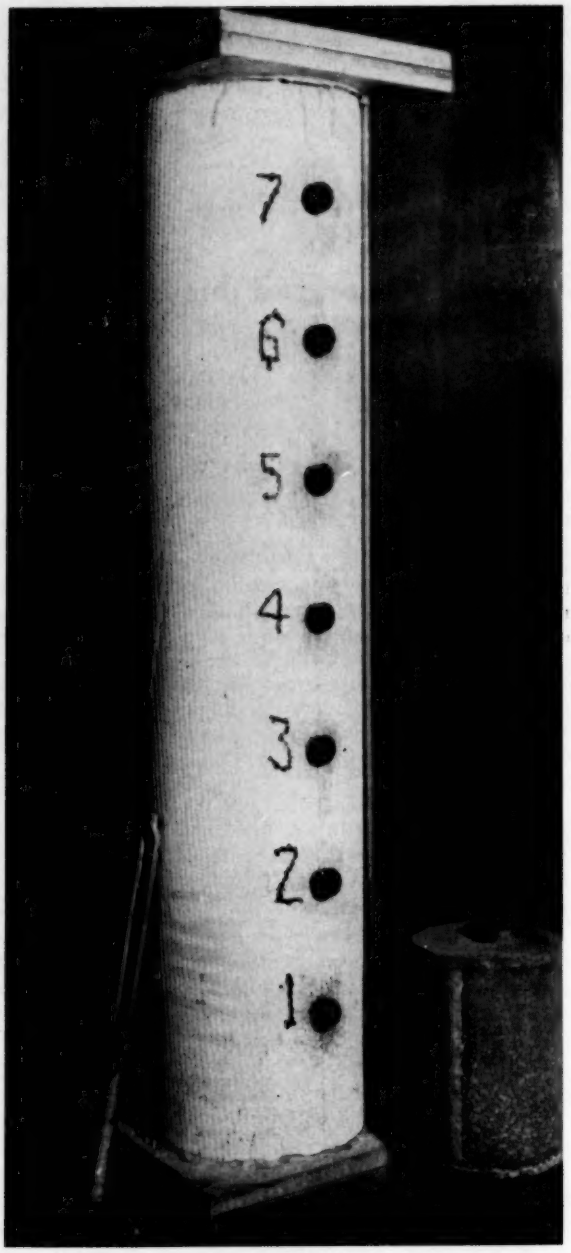


FIG. 1.—One of the cylinders in which the grain was stored; lower left, the sampling probe; lower right, the brass cloth cage filled with grain in which insects were confined at the bottom of two cylinders.

this was necessary with only two cylinders. Two of the cylinders served as controls, and no insects were added to them. To each of another pair, 200 adult granary weevils were added when the cylinders were half filled; then the remaining grain was added. To each of a third pair, 200 adult weevils were placed in each of two cylindrical cages made of 30-mesh brass cloth, each cage filled with 2.4 kg. of wheat through a hole in the top, and the hole closed with a rubber stopper. One cage was placed at the bottom of each cylinder, and 21 kg. of wheat were added. The aim was to permit moisture produced by the insects to move into the grain above. Figure 1 shows one of the cages.

Samples were withdrawn periodically from various ports by means of the probe shown in figure 1, and the probe was sterilized by flame and allowed to cool before each sample was taken. Each sample, immediately placed in a sterile test tube 1 inch in diameter, 6 inches in length and closed with a sterile rubber stopper, was usually tested within a few hours. The temperature of the grain was measured by inserting a thermistor probe to the center of the column at the desired ports.

Moisture content was determined by the two-stage, air-oven method specified by Cereal Laboratory Methods (American Association of Cereal Chemists 1957) and is expressed on a wet weight basis.

Colonies of fungi per gram of grain, or mold counts, were made as follows: 5 gm. of grain were put in 500 ml. of 0.15% sterile agar solution in a Waring Blendor, the blender run for 1½ minutes and 5 ml. of the suspension removed and put in 45 ml. of 0.15% sterile agar solution in a milk dilution bottle which was shaken briskly 100 times. Additional dilutions were made in the same way. Two 1-ml. portions of one or more dilutions were put into sterile petri dishes, and melted malt-salt agar (2% Difco powdered agar, 2% Difco powdered malt extract, and 7.5% to 10% sodium chloride) cooled to 52° to 54° C. was poured into the dishes which were swirled to distribute the suspended material uniformly. After the agar hardened, the dishes were stacked and incubated in the laboratory at room temperature. After 2 to 3 days, the dishes were inverted on the stage of a stereoscopic microscope and examined with transmitted light, using a magnification of 10×. The colonies were counted and the location of each one marked on the bottom of the dish with a wax pencil. The colonies were identified when they sporulated, usually after 5 to 7 days.

Surface-disinfected kernels yielding fungi were determined as follows: 50 or 100/kernels were shaken in 1% sodium hypochlorite for one-half minute, rinsed in sterile water, and cultured on malt-salt agar. Kernels from occasional samples were cultured also on water agar containing 18% sodium chloride, a medium recently found to be suitable for isolation and identification of *A. restrictus* from kernels in which other members of the *A. glaucus* group species may be present.

Germination percentage was determined by incubating 100 kernels on moist paper at room temperature for 3 to 5 days; any kernel that produced a sprout or root, even though stunted, was considered to have germinated.

RESULTS.—*Weevils Not Confined.*—The data from samples taken from these two cylinders are given in table 1. After 2 months, the weevils were concentrated near the bottom of the grain, although a few were found in the samples taken from the middle ports, and one was obtained from the sample taken from the top port of one cylinder. The moisture content of the grain at the bottom of the column, where the insects were most abundant, had increased slightly, but little or no change appeared in the fungus population.

After 4 months, the greatest population of weevils was found in the grain from the middle port, although considerable numbers were present near the bottom and a lesser number near the top of the grain. The moisture con-

**Table 1.—Changes in moisture content, temperature, and fungus population of wheat in a column 8 inches in diameter and 40 inches high, infested with 200 granary weevils and tested after 2, 4, and 6 months.**

| Port | Cylinder | Moisture Content (%) | Temperature (°C.) | Fungus Colonies/Gm. (thousands) *Aspergillus* | | Per Cent Surface Disinfected Kernels Yielding | | No. of Weevils[a] | | | Kernels Damaged (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *repens* | *restrictus* | *Alternaria* | *Aspergillus* | Alive | Dead | Total | |
| | | | | | *2 months* | | | | | | |
| 6 (top) | 1 | 13.1 | 23.4 | 0 | 0 | 46 | 2 | 1 | 0 | 1 | |
| | 2 | 13.0 | 23.5 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | |
| 4 (middle) | 1 | 13.3 | 23.3 | 0 | 0 | 36 | 0 | 6 | 0 | 6 | |
| | 2 | 13.0 | 23.2 | 0 | 0 | 60 | 0 | 2 | 0 | 2 | |
| 2 (bottom) | 1 | 13.6 | 23.6 | 0 | 0 | 50 | 0 | 16 | 0 | 16 | |
| | 2 | 13.4 | 23.7 | 0 | 0 | 36 | 0 | 20 | 0 | 20 | |
| | | | | | *4 months* | | | | | | |
| 6 | 1 | 14.8 | 26.8 | 2 | 0 | 16 | 2 | 27 | 0 | 27 | 13 |
| | 2 | 14.0 | 26.8 | 0 | 0 | 16 | 2 | 9 | 0 | 9 | 14 |
| 4 | 1 | 15.5 | 26.0 | 0 | 1 | 16 | 4 | 79 | 0 | 79 | 39 |
| | 2 | 15.9 | 25.8 | 0 | 0 | 10 | 0 | 44 | 0 | 44 | 18 |
| 2 | 1 | 16.0 | 25.3 | 552 | 18 | 2 | 34 | 34 | 0 | 34 | 26 |
| | 2 | 15.6 | 24.8 | 0 | 3 | 16 | 0 | 38 | 0 | 38 | 15 |
| | | | | | *6 months* | | | | | | |
| 6 | 1 | 20.2 | 29.0 | 1,850 | 19,200[b] | 0 | 100 | 3 | 26 | 29 | 62 |
| | 2 | 19.2 | 28.0 | 2,750 | 18,500[b] | 0 | 100 | 6 | 35 | 41 | 70 |
| 4 | 1 | 17.3 | 27.8 | 1,730 | 28,050[b] | 0 | 100 | 4 | 43 | 47 | 60 |
| | 2 | 15.8 | 26.2 | 250 | 13,000 | 0 | 100 | 11 | 36 | 47 | 60 |
| 2 | 1 | 16.1 | 26.2 | 1,350 | 24,300 | 0 | 100 | 11 | 74 | 85 | 74 |
| | 2 | 15.6 | 25.5 | 200 | 37,800 | 0 | 100 | 5 | 39 | 44 | 46 |

[a] Number of weevils found in a 25-gm. sample of grain.
[b] These yielded also from 300,000 to 8,000,000 colonies/gm. of *Spicaria*.

tent of the grain was greatest near the bottom of the column, where the insects evidently had been most active in the period between 2 and 4 months, slightly lower in the center of the column, and still lower at the top where it had, however, increased approximately 1%. The temperatures were lowest at the bottom of the grain column and highest at the top. The greatest increase in temperature was approximately 6° C. (10.8° F.). No measurements or calculations were made to determine the amount of heat lost from the grain during the course of the tests. It is probable that much more heat was dissipated from these small bulks than would be lost with an equally heavy infestation in larger bulks in commercial storage. However, it appears that even a slight rise in temperature may indicate a moderately developed infestation (port 2, cylinder 1, 4 months, in table 1) and fairly well advanced invasion by storage fungi, in that portion of the grain where the temperature is rising. *A. repens* had increased greatly in the grain at the bottom of cylinder No. 1, and *A. restrictus* had developed in the grain at the bottom of both cylinders. *Alternaria*, a field fungus present in more than 75% of the original kernels, had largely disappeared and was being replaced by *Aspergillus*.

After 6 months, the population of living insects had decreased in all the samples, but judging from the percentage of kernels damaged by insects, the infestation had been fairly heavy throughout the columns. Moisture content was highest (approximately 20%) in the grain near the top of the column and decreased toward the bottom; the same was true of temperature. The population of storage fungi, especially of *A. restrictus*, was exceedingly high, and *Alternaria* had disappeared from the grain. The increase in moisture content and in storage fungi throughout the grain as a whole in 6 months was much greater than reported in previous tests (Agrawal *et al.* 1958), almost certainly because in the present tests the loss of water from the grain was kept to a minimum.

*Insects Confined in Cages at the Bottom of the Cylinders.*—The results of tests on samples taken from these two cylinders are summarized in table 2. The moisture content in the grain just above the confined insects increased gradually, and after 6 months was 2% to 3% higher than originally. Storage fungi, especially *A. restrictus*, increased greatly in this portion of the grain (fig. 2), and the germination percentage of the wheat decreased to almost zero. Between the fourth and sixth months, the moisture content in the grain showed some increase at the middle of the column, and a slight increase in moisture content in the grain near the top, about 30 inches above the portion where the insects were confined. That this increase in moisture content in grain at the middle of the column was real, and not owing to chance variation in moisture content determinations, is indicated by the fact that the count of storage fungi also increased in the grain

**Table 2.—Changes in moisture content, fungus population, and germination percentage of wheat in a column 8 inches in diameter and 40 inches high, with weevils confined in 2.4 kg. of grain at bottom of column.[a]**

| Port | Cylinder | Moisture Content (%) | Fungus Colonies/Gm. (thousands) | | Per Cent of Surface Disinfected Kernels Yielding | | Germination (%) |
|---|---|---|---|---|---|---|---|
| | | | *Aspergillus* | | | | |
| | | | *repens* | *restrictus* | *Alternaria* | *Aspergillus* | |
| | | | *2 months* | | | | |
| 6 (top) | 1 | 13.7 | 0 | 0 | 68 | 0 | 89 |
| | 2 | 13.3 | 0 | 0 | 64 | 0 | 93 |
| 4 (middle) | 1 | 13.8 | 0 | 0 | 55 | 0 | 97 |
| | 2 | 13.6 | 0 | 0 | 48 | 0 | 88 |
| 2 (bottom) | 1 | 13.9 | 0.5 | 0 | 58 | 2 | 96 |
| | 2 | 13.9 | 0.5 | 0 | 53 | 4 | 89 |
| | | | *4 months* | | | | |
| 6 | 1 | 12.8 | 0.5 | 2 | 30 | 2 | 85 |
| | 2 | 13.0 | 0.5 | 0 | 38 | 14 | 94 |
| 4 | 1 | 13.7 | 2 | 0 | 28 | 10 | 92 |
| | 2 | 13.5 | 2 | 2.5 | 18 | 26 | 90 |
| 2 | 1 | 14.1 | 1.5 | 4.5 | 22 | 26 | 91 |
| | 2 | 14.1 | 27 | 17 | 10 | 42 | 83 |
| | | | *6 months* | | | | |
| 6 | 1 | 13.7 | 0.5 | 2.5 | 20 | 4 | 96 |
| | 2 | 13.5 | 2.5 | 3 | 10 | 2 | 95 |
| 4 | 1 | 14.1 | 2 | 4 | 10 | 8 | 95 |
| | 2 | 14.1 | 3.5 | 3 | 14 | 14 | 94 |
| 2 | 1 | 15.1 | 50 | 6,950 | 6 | 46 | 16 |
| | 2 | 16.8 | 5.5 | 15,900 | 0 | 76 | 2 |
| | | | *Controls, 6 months* | | | | |
| 6 | 1 | 13.4 | 0 | 0 | 6 | 0 | 30 |
| | 2 | 13.1 | 0 | 0 | 2 | 0 | 94 |
| 4 | 1 | 13.5 | 0 | 0 | 2 | 0 | 76 |
| | 2 | 13.1 | 0 | 0 | 10 | 0 | 95 |
| 2 | 1 | 13.4 | 0 | 0 | 8 | 0 | 87 |
| | 2 | 13.1 | 0 | 0 | 8 | 0 | 96 |

[a] Tested after 2, 4, and 6 months.

at the middle of the column. Under conditions that permit only a slow escape of moisture vapor from bulk grain, a weevil infestation evidently can contribute to increased moisture content and increased development of storage fungi in a bulk considerably larger than that occupied by the insects.

*Controls.*—Test data for the controls after storage for 6 months are given at the bottom of table 2. The data from tests of the samples from the controls after 2 and 4 months are not included, since there was essentially no change during the storage period. *Alternaria* gradually disappeared from the grain in the control cylinders, as was to be expected, and germination percentage of the grain from cylinder No. 1, which had a slightly higher moisture content than the grain in cylinder No. 2, decreased, but no increase in storage fungi was detected.

*Effect of Fumigation on Storage Fungi.*—Shortly after samples were taken at the end of the 6-month storage period, the grain was fumigated with a commercially used mixture of carbon tetrachloride, ethylene dichloride, and ethylene dibromide[3] at the rate of 3 ml. to approximately three-fourths bushel of grain. The cylinders were kept sealed for 24 hours after the fumigant was added, then ventilated. Subsequent sampling indicated that all immature and adult weevils were killed. Four days after fumigation, and again 2 months after fumigation, samples were taken from the top, middle, and bottom ports of each cylinder and tested as before, the major aim being to determine whether the fumigant had had any effect on the population of storage fungi. Table 3 shows data on the population of *A. restrictus* before and after fumigation in the grain from the middle ports of the pair of cylinders in which the insects had been allowed to wander freely, since these were typical of the results obtained from the other samples similarly taken and tested. Figure 3 illustrates

[3] "Hot Spot" fumigant formulated by the E. H. Leitte Co., Minneapolis, Minnesota.

FIG. 2.—*Aspergillus restrictus* sporophores growing from the germs of grain taken from port #2, above the cage in which insects were confined; after storage for 6 months.

the increase of fungi.

No detectable effect of fumigation on the storage fungi appeared. All the samples with a large population of storage fungi before fumigation had, after fumigation, at least some millions of viable spores per gram of grain, and within 2 months after fumigation, the population had increased considerably. There probably was less circulation of air through the grain in these rather tightly sealed cylinders than there would be in most commercial bins. Therefore, if fumigant adsorbed on the grain were expected to have an inhibiting effect on storage fungi, it should have been apparent here. It seems fair to suppose that fumigation as ordinarily practiced in commercial bins of grain will have relatively little deterrent effect upon storage fungi. Where the moisture content of the grain before fumigation is high enough to permit development of storage fungi, as it is likely to be in grain with a well-developed infestation by granary weevils and in a bulk of grain above the infestation at least equal to or somewhat greater than the bulk inhabited by the insects, the storage fungi may be expected to continue to develop after fumigation. In support of this supposition, several samples of wheat were obtained during the past year from commercial bins in which infestations of *S. granarius* had developed, accompanied by vigorous development of storage fungi. These bulks had been fumigated shortly before the samples were taken, and no living insects were found in them. Dilution cultures of these samples yielded from several hundred thousand to several million colonies of storage fungi per gram of grain, principally *A. restrictus* and various other members of the *A. glaucus* group, but in one case various other species of *Aspergillus*.

**Table 3.—Effect of fumigation on the population of *Aspergillus restrictus* in wheat.**

| TIME OF SAMPLING | *Aspergillus restrictus* COLONIES/GRAM OF WHEAT (THOUSANDS)[a] | MOISTURE CONTENT (%) |
|---|---|---|
| Before fumigation | 20,000 | 16.5 |
| 4 days after fumigation | 24,000 | 15.8 |
| 2 months after fumigation | 51,000 | 16.5 |

[a] Each figure is an average of two samples, one from the middle port of each of the duplicate cylinders in which the weevils were not confined.

The grain used in these tests was of seed grade. It recently has been shown (Papavizas & Christensen 1959, Wyllie & Christensen 1959) that different lots of wheat stored under essentially identical conditions and even when inoculated with the same species of storage fungi may deteriorate at greatly different rates. In the column of grain with insects confined at the bottom, the increase in moisture content, in the present tests, extended consid-

FIG. 3.—Grain from the same port as that shown in figure 2, 2 months after the grain had been fumigated to kill the insects. Sporulation of *Aspergillus restrictus* is considerably heavier than it was before fumigation.

erably higher than in the tests reported by Agrawal *et al.* (1958). Had commercial samples of wheat been used in the present tests instead of seed grade, it is possible that this increase in moisture content throughout much of the column might have been accompanied by a larger increase in storage fungi and greater deterioration farther above the confined insects. Tests to determine this premise now are under way with similar but larger cylinders and grain of grade No. 2 from commercial bins.

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## NOTICE TO MEMBERS

Authors are urged to consult the latest revised list of "Common Names of Insecticides." See JOURNAL OF ECONOMIC ENTOMOLOGY **52**(2): 361–362. April 1959. Reprints are available free upon request to the Office of the Executive Secretary.

The chemical definition of each insecticide, the name of which does not appear in this list, should be given the first time the name occurs in a manuscript accepted for publication by the Entomological Society of America; also in the abstract.

# The Suitability of Tobaccos for the Growth of the Cigarette Beetle, *Lasioderma serricorne*[1]

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ABSTRACT

Of four varieties of tobaccos tested, the cigarette beetle (*Lasioderma serricorne* (F.)) developed well on flue-cured, very slowly and with high mortality on Turkish and Maryland, and not at all on burley. This response had no direct relationship to the nicotine content, which was 1.72%, 1.02%, 1.56% and 4.17%, respectively. Growth was greatly stimulated by the addition of yeast or B-vitamins, but not by that of casein, glucose, or cholesterol. Major characteristics of insects reared on the various types of tobacco fortified with yeast were: 1) optimal growth and survival on flue-cured; 2) slower growth and smaller adults, but good survival on Turkish and Maryland; and 3) slow growth, small adults, and high mortality on burley. On a flour-plus-yeast diet nicotine up to 10% was well tolerated while it was harmful to two other insects, *Tribolium* and *Tenebrio* at 0.1%. In tobaccos, however, the tolerance was below 4%. It was concluded that the suitability of tobaccos for *Lasioderma* was dependent on: 1) the nicotine content; 2) the adequacy of nutrients; and 3) material which enhances the toxicity of nicotine.

Though the cigarette beetle, *Lasioderma serricorne* (F.), infests stored tobaccos frequently, and at times quite heavily, observations so far indicate that all tobaccos are not attacked indiscriminately or with equal intensity. Thus Smith (1907), Jones (1913), and Runner (1917) reported that under storage conditions the milder, higher grade, light-colored tobaccos were infested more readily than the poorer, filler grade, dark-colored tobaccos. In addition, they noted that the insect grew more rapidly and attained a larger size on the better grades than on the poorer grades of tobacco. Powell (1931), by rearing the insect on various types and grades of tobacco, also observed these characteristic growth differences while Reed & Vinzant (1942) reported that this beetle damaged all principal types of cigar, snuff, and cigarette tobaccos except burley and Maryland.

*Lasioderma* is not restricted to tobaccos and has been reported to infest a wide variety of stored and manufactured products (Runner 1917). The ability to thrive on seemingly poor food materials may be due, in part, to its symbiotic relationship with certain yeasts which provide eight vitamins of the B-complex group as well as sterols (Blewett & Fraenkel 1944, Pant & Fraenkel 1950, 1954). Insofar as tobaccos are concerned, Vinzant (1954) communicated that the insect thrived best on flue-cured and Turkish tobaccos and further expressed the opinion that nicotine rather than sugars appears to limit its growth.

These observations show sufficient indications that the variable growth responses of *Lasioderma* may be due to qualitative or quantitative differences in the composition of the tobaccos. In an analysis of four types of tobacco, Darkis & Hackney (1952) gave values for certain constituents that varied widely from type to type. For example, two tobacco types had maximal sugar contents of 32% and 19% while the other two had maximals of only 1.5%. By analyzing the effect of such compositional differences, some of the observed phenomena such as the large or small size attained by the insects, their rapid or slow growth, and the infestation or noninfestation of certain tobaccos may be explained. The study reported herein was undertaken, therefore, to compare, on the basis of certain growth criteria, the relative fitness of four types of tobaccos as a food for *Lasioderma* in respect to their nutritional and toxic qualities.

METHODS AND MATERIALS.—Stock cultures of the insect were maintained on whole wheat flour +5% brewer's yeast. Eggs were collected by placing newly emerged adult beetles on a mixture of white flour and brewer's yeast, and by sifting the beetles and flour through several different screen sieves at 2-day intervals. The eggs were then placed in 1-inch shell vials until they hatched.

The tobacco diets consisted of four different types of tobacco:[2] flue-cured, which was light yellow, and Turkish, Maryland, and burley, which were light brown to dark brown. The tobaccos were prepared for the dietary studies by oven drying the leaves at 65° C. for 1 hour and then grinding them in a mortar until they passed through a 40-mesh screen. One gram of each type of tobacco constituted the basic diet, to which other materials were added. All diets were mixed thoroughly in mortars, placed in 1-inch shell vials, and equilibrated in a cabinet at 30° C. and 65±5% R. H. for 2 days. Ten newly hatched larvae were then placed in each vial and the vial capped with a cloth cover. All tests were run in duplicate. The growth of the insects on the various diets was evaluated on the basis of the number, the time to emergence and the weight of the adults.

In the nontobacco diets containing various percentages of casein and glucose, cellulose comprised the balance of the substrate. Five per cent yeast was added to these stock diets.

Nicotine was incorporated in the tolerance-test diets in the form of nicotine bitartrate and was analyzed by the silicotungstic acid method for small samples (Avens & Pearce 1939).

EXPERIMENTS AND DISCUSSION.—Growth on whole wheat flour+5% yeast was considered to be normal and was made the standard in evaluating the other diets. Since the total available nutrients were reported to vary considerably from one type of tobacco to another, the effects of high and low amounts of glucose and casein were first tested in cellulose diets. These results are recorded in table 1. Subsequently, the effect of supplementing the different tobaccos with casein, glucose, yeast, vitamins, and cholesterol was tested (table 2).

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[2] The tobaccos were obtained through courtesy of the American Tobacco Company, Richmond, Virginia.

**Table 1.—Growth of the cigarette beetle on artificial diets and on whole wheat flour.[a]**

| DIET | NUMBER OF ADULTS | AVERAGE TIME TO EMERGENCE OF ADULTS (DAYS) | AVERAGE WEIGHT PER ADULT (MILLIGRAMS) |
|---|---|---|---|
| 50% casein+50% glucose+5% yeast | 20 | 27.1 | 2.10 |
| 25% casein+25% glucose+5% yeast+50% cellulose | 18 | 28.9 | 1.81 |
| 10% casein+25% glucose+5% yeast+65% cellulose | 19 | 30.3 | 1.74 |
| 10% casein+10% glucose+5% yeast+80% cellulose | 17 | 28.7 | 1.83 |
| 5% casein+5% glucose+2½% yeast+90% cellulose | 20 | 32.5 | 1.60 |
| 25% casein+5% yeast+75% cellulose (no glucose) | 19 | 30.4 | 1.52 |
| 10% casein+5% yeast+90% cellulose (no glucose) | 18 | 32.9 | 1.56 |
| Whole wheat flour+5% yeast | 18 | 27.5 | 2.34 |

[a] Twenty first instar larvae used per test diet.

**Table 2.—Growth of the cigarette beetle on four types of tobacco.[a]**

| ADDITIONS | FLUE CURED | | | TURKISH | | | MARYLAND | | | BURLEY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Average | | | Average | | | Average | | | Average | |
| | No. of Adults | Emergence Time (Days) | Weight (mg.) | No. of Adults | Emergence Time (Days) | Weight (mg.) | No. of Adults | Emergence Time (Days) | Weight (mg.) | No. of Adults | Emergence Time (Days) | Weight (mg.) |
| No addition | 13 | 43.6 | 1.81 | 1 | 72.0 | 0.89 | — | — | — | — | — | — |
| 5% yeast | 19 | 32.1 | 2.13 | 15 | 35.0 | 1.10 | 18 | 37.0 | 1.37 | 5 | 43.8 | 1.03 |
| Vitamin solution 2× strength | 19 | 31.7 | 1.95 | 5 | 44.0 | 1.20 | 1 | 50.0 | 1.25 | — | — | — |
| Vitamin solution 4× strength | 18 | 31.2 | 1.96 | 2 | 39.0 | 1.00 | 4 | 44.5 | 1.40 | — | — | — |
| 10% casein+10% glucose | 15 | 37.6 | 2.16 | 6 | 48.2 | 1.60 | 2 | 49.5 | 1.35 | — | — | — |
| 10% casein+10% glucose +0.1% cholesterol | 14 | 37.0 | 2.09 | — | — | — | 4 | 48.0 | 1.23 | — | — | — |
| 10% casein+10% glucose +5% yeast | 18 | 34.0 | 2.12 | 15 | 33.3 | 1.29 | 17 | 37.2 | 1.12 | 4 | 55.2 | 1.00 |

[a] Twenty first instar larvae used per test diet.

**Table 3.—The effect of dietary nicotine on the growth of three species of stored products insects.[a]**

| PER CENT CONCENTRATION OF NICOTINE IN WHOLE WHEAT FLOUR+5% YEAST | *L. serricorne* | | | *Tribolium confusum* | | *Tenebrio molitor* | |
|---|---|---|---|---|---|---|---|
| | | Average | | | | | |
| | No. of Adults | Time to Emergence (Days) | Weight (mg.) | No. of Pupae | Average Time to Pupation (Days) | No. of Larvae | Weight After 4 Weeks (mg.) |
| None (Control) | 19 | 27.7 | 1.93 | 18 | 15.6 | 18 | 8.40 |
| 0.01 | — | — | — | 17 | 17.5 | 19 | 8.50 |
| 0.025 | — | — | — | 18 | 21.0 | 14 | 9.50 |
| 0.05 | — | — | — | 11 | 23.8 | 10 | 6.00 |
| 0.1 | — | — | — | 4 | 25.5 | 5 | 3.58 |
| 0.25 | 16 | 26.4 | 2.03 | 0 | 0 | 7 | 2.58 |
| 0.5 | 15 | 27.8 | 1.71 | 0 | 0 | 2 | 2.60 |
| 1.0 | 19 | 29.7 | 1.88 | — | — | 4 | 2.89 |
| 2.0 | 16 | 29.2 | 1.79 | — | — | 0 | 0 |
| 4.0 | 17 | 31.6 | 1.81 | — | — | 0 | 0 |
| 6.0 | 16 | 32.2 | 1.56 | — | — | — | — |
| 10.0 | 16 | 34.2 | 1.70 | — | — | — | — |

[a] Twenty first instar larvae of each species used at concentrations of nicotine indicated.

Nicotine concentrations of the tobaccos dried to constant weight were burley 4.17%, flue-cured 1.72%, Maryland 1.56% and Turkish 1.02%. To test whether nicotine in the concentrations present in the tobaccos may have affected the growth of the insect, whole wheat flour+5% yeast diets were prepared in which nicotine was added at levels from 0.25% to 10%. Two other stored grain insects, *Tenebrio molitor* L. and *Tribolium confusum* Duv. were employed as controls. The high degree of tolerance to nicotine by *Lasioderma* is shown in table 3, and table 4 shows the adverse effect of elevating the nicotine content in a good tobacco diet.

From the results of the experiments, growth of *Lasioderma* on nutrient supplemented tobaccos can be grouped into three categories as follows: 1) rapid growth and nearly normal adults on flue-cured tobacco; 2) slow growth and small adults on Turkish and Maryland; and 3) slow growth, small adults and high mortality on burley tobacco (table 2).

In general, the variable growth responses of *Lasioderma*

Table 4.—The effect of increased nicotine concentration in flue-cured tobacco on the growth of the cigarette beetle.[a]

| PER CENT CONCENTRATION OF NICOTINE | NUMBER OF ADULTS | AVERAGE TIME TO EMERGENCE (DAYS) | AVERAGE WEIGHT (MG.) |
|---|---|---|---|
| 1.72 (Normal) | 16 | 35.5 | 1.63 |
| 2.0 | 18 | 38.2 | 1.23 |
| 2.5 | 11 | 42.5 | 1.37 |
| 3.0 | 12 | 38.3 | 1.38 |
| 3.5 | 11 | 41.9 | 1.07 |
| 4.0 | 5 | 40.4 | 1.20 |
| 4.5 | 6 | 46.5 | 1.22 |
| 5.0 | 8 | 40.6 | 1.19 |
| Whole wheat flour +5% yeast (Control) | 16 | 34.4 | 1.75 |

[a] Twenty first instar larvae used for each test diet. All the diets contained 5% yeast.

listed above may reflect the disuniformity in the composition of the different types of tobacco. These compositional differences appear to depend to a large extent on the curing and fermentation processes. In the curing of tobaccos, the breakdown of the major components of green leaves, such as carbohydrates, chlorophyll, organic acids, et cetera, occurs in the early stages while the moisture content and the activity of the cells are still quite high (Vickery & Meiss 1953). However, as the methods and the length of time involved in the curing processes, such as fire-curing, flue-curing and sun-curing differ, the final compositions of the tobaccos undergoing the different processes vary considerably (Darkis & Hackney 1952).

Of the essential nutrients, the curing process appears to degrade the B-vitamins largely, since in the tests the addition of small amounts of yeast, and to a lesser extent a solution of B-vitamins used commonly in this laboratory in dietary studies of grain insects (Fraenkel *et al.* 1950) were immensely effective in stimulating growth (table 2). On flue-cured tobacco, the addition of the vitamins alone was sufficient to give growth comparable to that given on whole wheat flour+5% yeast, and on Turkish, Maryland, and burley tobaccos, yeast was effective in promoting growth where little or none occurred in its absence. It appears, therefore, that flue-cured is deficient solely in B-vitamins, while the other three varieties are lacking, in addition, other nutrients supplied by yeast. However, even with yeast added, growth on the latter three tobaccos was still characterized by prolonged growth periods and reduced size of the adults.

The total protein and sugar contents may determine the size of the adults: The gradual diminution from a high to a low concentration of both components resulted in a corresponding reduction in the weights of the beetles (table 1). The total absence of sugars also resulted in smaller beetles, but in no case was mortality affected in these diets. However, assumptions that low concentrations of either sugars or proteins may account for the small size of the insects obtained on three of the tobaccos (Maryland, Turkish, and burley) were not supported by the results. The addition of casein and glucose along with yeast gave no improvement over the addition of yeast alone (table 2), and when compared with the cellulose diet of 10% casein-10% glucose-5% yeast, growth on these tobaccos with the identical adjuvants was much inferior.

Consideration of factors other than nutrients that may account for the prolonged growth and diminutive adults in tobacco insects led to the analysis of nicotine and the measurement of its effect on *Lasioderma*. Tolerance to dietary nicotine had been expected at levels commonly found in tobaccos, but not at the high levels shown in table 3. While the control insects, *Tenebrio* and *Tribolium*, had high mortalities at a nicotine concentration of only 0.1%, *Lasioderma* grew normally at this concentration and tolerated higher concentrations up to 10% with only slight reductions in weight and prolongation of the growth period. This high tolerance to nicotine in no way accounted for the poor growth obtained on burley tobacco, which contained slightly more than 4% nicotine. This fact suggested the presence of other materials in tobaccos, which may have enhanced the toxicity of nicotine.

This suggestion was borne out when the nicotine content of flue-cured tobacco, which gave nearly normal growth when supplemented with yeast, was elevated to the level found in burley tobacco (4%) (table 4). The high mortality, the small size, and the prolonged growth of the insects on flue-cured tobacco at the 4% nicotine level and above closely paralleled the results on burley tobacco supplemented with yeast. Nicotine at higher concentrations is far more toxic in tobacco diets that had been adequately supplemented with nutrients, than it is on a flour-yeast diet. On the other hand, insects reared on the low nicotine Turkish and Maryland tobaccos, with nutrients added, did not suffer any mortality, but were far smaller than those raised on the corresponding flue-cured tobacco diets. Since these three tobaccos have the same low nicotine content, and since all the diets were made nutritionally adequate, the Turkish and Maryland diets must have contained some other harmful material.

Growth of the cigarette beetle on tobacco, therefore, appears to depend on at least three factors, namely the adequacy of the nutrients, nicotine, and a material which enhances the effect of nicotine. All the tobaccos tested were deficient in nutrients, among which the B-vitamins appeared to play the most important role. Nicotine was lethal only at the higher concentrations, as in burley, and its effect was enhanced by some other material present in tobaccos. Whether or not this nicotine-enhancing material is identical with the material in Turkish and Maryland tobaccos, mentioned above, which renders Turkish and Maryland inferior to flue-cured, cannot be decided on the evidence available. It is conceivable that this material is present in flue-cured tobacco in lower concentrations.

These results allow the conclusions that of the tobaccos tested, only flue-cured would be highly susceptible to *Lasiodema serricorne*. This susceptibility can be ascribed to its low nicotine content, its better nutritional quality, and possibly a lower content of a nicotine-enhancing substance.

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# Citrus Red Mites Resistant to Demeton and Ovex and Their Response to Tedion and Kelthane[1]

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ABSTRACT

Strains of citrus red mites (*Panonychus citri* (McG.)) that had become resistant to demeton and ovex by repeated spray treatments were collected for laboratory rearing and testing. The demeton-resistant mites, which were tested in the mature stage on green lemons, treated by dipping, were found to be 163 times as resistant as a nonresistant strain. The ovex-resistant mites were 131 times as resistant, as measured by the number that developed to maturity from a given number of eggs on treated lemons. No cross-tolerance to Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) or Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) existed in either strain. Tedion was about 10 times as effective as Kelthane.

Repeated applications of demeton and ovex against the citrus red mite (*Panonychus citri* (McG.)) in California have created strains resistant to these compounds (Jeppson *et al.* 1958). In order to study the stability of resistance to these acaricides in the absence of treatment, resistant strains were collected in the field and brought to the laboratory for rearing without further exposure to chemicals. This paper reports the initial resistance to demeton and ovex and the tests for cross-tolerance to Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) and Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical).

REARING PROCEDURE.—The progeny of mites from a single collection of each strain were used. The different strains were reared continuously in the laboratory in the manner previously described (Munger 1957), with the following modifications: The mites were reared on green lemons held on nail racks in a room maintained at 75° F. and 60% relative humidity. A more efficient miteproof cover was substituted for the described flannelette wrapping to isolate the different strains. The cover consisted of a 5-inch-tall wooden frame 8×15 inches, made of 3/4-inch pine, with 3/16-inch felt glued to the top and bottom edges. A plate-glass cover allowed observation and made the mites more accessible (fig. 1). Ventilation was provided by muslin-covered holes in the sides and ends of the frame.

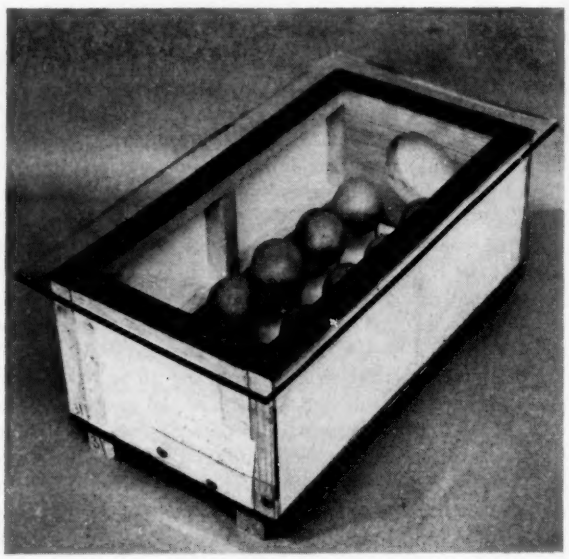

FIG. 1.—Ventilated cover for isolating strains of citrus red mites in rearing unit.

Mass transfers of mature females from source lemons to obtain eggs were made by means of rubber collars approximately 7/8 inch tall by 1 5/8 inches in diameter. The collars, notched at top and bottom to prevent trapping of mites, were placed on the upper side of the source lemons. Fresh lemons were then placed on the collars and left for 1

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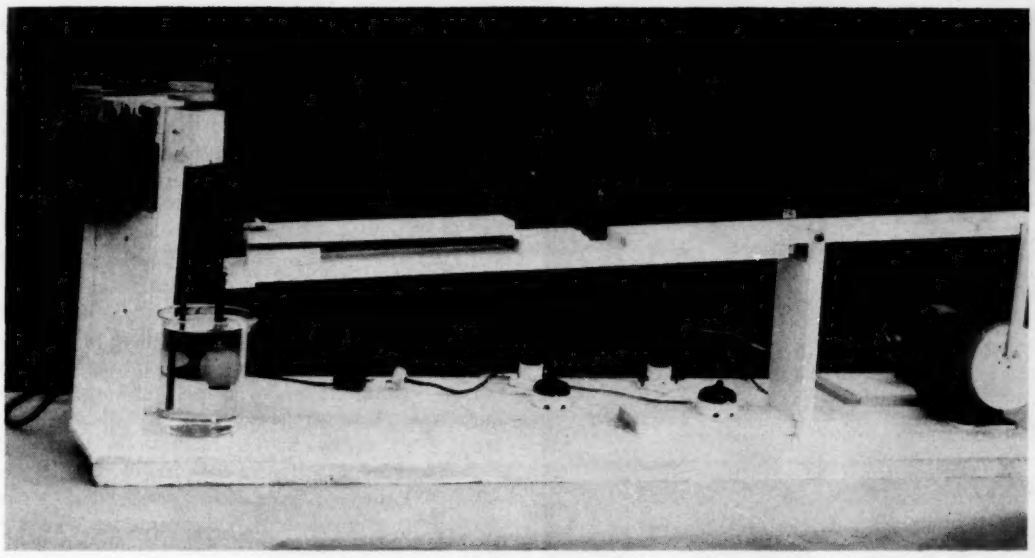

FIG. 2.—Apparatus for dipping test lemons in acaricides.

day, or until enough mites transferred. This method worked well only when the mites were crowded and needed fresh food. In an alternative method, a strip of tissue paper was laid on the nail rack before the lemons were placed. The mites were then brushed from the source lemons. Most of them landed on the paper, from which they found their way to the new lemons. After 2 to 4 days, when sufficient eggs had been deposited, the mites were either washed off under a water tap, or brushed off to be used again. The lemons were then washed. The surface of the lemon, except for eggs, is cleaned by washing. The lemons were then placed in the rearing room for development of the new generation.

ACARICIDE APPLICATION.—The acaricides were applied to the lemons by dipping. A device (fig. 2) that standardized the dipping time consisted of a lever operated by a motor-driven eccentric and a clamp at the dipping end to hold a lemon. The lemons were immersed one at a time in a liter of water suspension or emulsion of agitated material. The period of immersion was 5.5 seconds. They were then held on nail racks to dry.

To measure the amount of acaricide deposited, a series of lemons were dipped in eight concentrations of ovex and the dried deposits were determined as total organic chlorine by the sodium reduction method. Results are shown in table 1. Deposits increased with the concentration, but the relative adherence, as shown in the ratio of deposit to concentration, tended to decrease at high concentrations.

NONRESISTANT STRAIN.—The nonresistant strain of mites was collected in January 1952 from a lemon orchard near Brea and has been reared continuously since that time, first in the insectary and later in the laboratory. The grove from which it was collected had an oil-treatment history, except for one application of parathion. The susceptibility of the laboratory strain was about the same as that of a strain from an abandoned orchard with an oil history near San Juan Capistrano. The nonresistant strain used by Jeppson *et al.* (1958) was also collected from this orchard.

RESISTANCE TO DEMETON.—A collection of demeton-resistant mites was made in July 1957, at Corona, in a lemon grove of the Foothill Lemon Company. This strain has been reared continuously in the laboratory. The grove from which the collection was made had received six demeton treatments from 1954 through 1956, plus an application of parathion each spring, followed by Neotran® (bis(*p*-chlorophenoxy)methane) treatments in 1956 and 1957. Before the demeton treatments, the block had been under an ovex program for 3 years.

Newly emerged female mites were used for tests. Demeton was applied in an emulsion. After being dipped and dried, the lemons were placed stem end down in stiff, plastic funnels 2 3/4 inches in diameter and 2 3/4 inches tall. The funnels were then placed in tumblers of water (fig. 3). The water acted as a barrier to restrict the mites to the lemons (Metcalf 1948). Twenty-four hours after treatment, 20 recently-emerged mature females were transferred by hand to each of five lemons for each concentration of acaricide. Mortality counts, based on the number of surviving mites, were made 48 hours later. The funnels with lemons in place were lifted from the tumblers to avoid crushing the mites and because of the danger of handling demeton-treated fruit.

**Table 1.—Ovex deposits on lemons treated by dipping.[a]**

| CONCENTRATION (GRAMS/LITER) | NUMBER OF LEMONS | DEPOSIT (MICROGRAMS/SQ. CM.) | RATIO OF DEPOSIT TO CONCENTRATION |
|---|---|---|---|
| 0.125 | 20 | 0.45 | 3.6 |
| .25 | 20 | .88 | 3.5 |
| .50 | 10 | 1.84 | 3.7 |
| 1.00 | 15 | 3.73 | 3.7 |
| 4.00 | 13 | 13.42 | 3.4 |
| 6.36 | 9 | 16.93 | 2.7 |
| 10.11 | 9 | 27.55 | 2.7 |
| 16.08 | 9 | 44.86 | 2.8 |

[a] Chemical analyses were made by Robert P. Muns, Entomology Research Division, Agric. Res. Serv., Whittier, California.

FIG. 3.—Test lemon in funnel and tumbler.

Dosage-mortality curves for three tests comparing resistant and nonresistant strains are shown in figure 4. The regression lines were calculated according to the method of Finney (1952). The slope averaged 3.74 for both strains. A comparison of the LD50s between strains is shown in table 2. The relative potencies from one test to another were within the limits of error. Jeppson *et al.* (1958), working with freshly collected mites from another source, found them to be 266 and 192 times more resistant at LD50 and LD95, respectively, than nonresistant mites.

RESISTANCE TO OVEX.—Ovex-resistant mites were collected from the Limoneira Ranch at Santa Paula in August 1958. The block from which these mites were taken had been under an intensive ovex program, which included combinations with other materials such as petroleum oil, Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), and Dow DN 111 (the dicyclohexylamine salt of 4,6-dinitro-2-cyclohexylphenol), from 1951 through the spring of 1957. The ovex-resistant mites were compared with the same nonresistant strain used in the demeton studies.

Ovex was believed to act primarily as an ovicide, but in preliminary tests the eggs of the resistant strain proved to be so resistant that the heavy deposits required to cause even low mortalities made counting very difficult. The highest mortality was 42.7% at a dosage of 53.24 grams per liter. Probably an increase in the deposit would not have produced substantially higher mortality in this strain. Further tests indicated that, although heavy deposits had little effect on the mature females, immature mites were susceptible. It was therefore believed that a better measure of toxicity of this material might be ob-

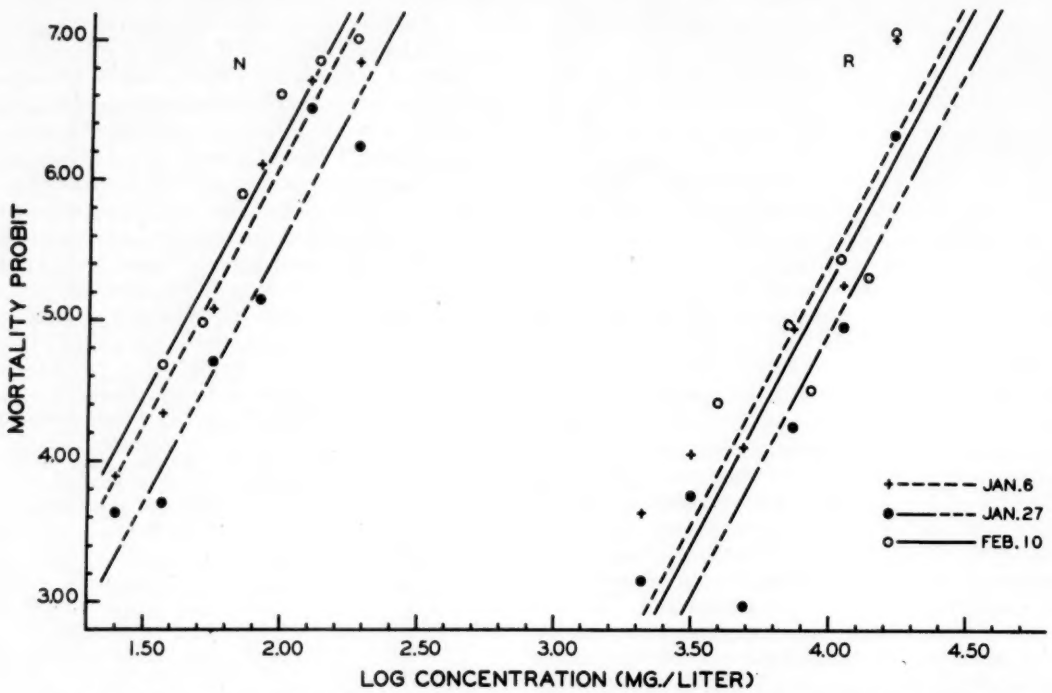


FIG. 4.—Mortality of nonresistant (N) and demeton-resistant (R) citrus red mites treated with demeton. Comparisons were made between the two strains on dates indicated.

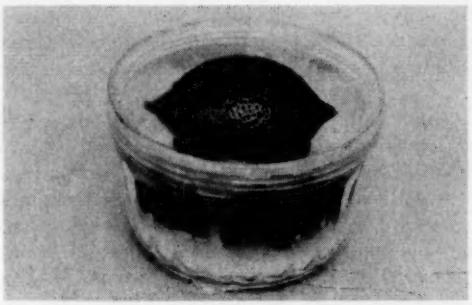

FIG. 5.—Lemon partially immersed in water in jelly glass to obtain citrus red mite eggs in a restricted area.

tained from the number of mites that matured from a given number of eggs. Preliminary tests gave satisfactory results, and the method was adopted.

To obtain eggs in a restricted area of the lemons, the fruits were placed in low jelly glasses partially filled with water (fig. 5), to limit the area available for oviposition. The lemon was fastened with paraffin to a metal collar imbedded in paraffin at the bottom of the glass, to stabilize the lemon and prevent the mites from being accidentally submerged at the edge of the water.

Thirty mites were placed on each lemon and allowed to oviposit for approximately 24 hours. A small India ink mark was made on the surface near each egg. The lemons were then removed from the jelly glasses, washed under the tap to remove the adult mites, and placed on nail racks. After 4 days in the rearing room, or 1 or 2 days before the eggs began to hatch, the lemons were dipped in ovex suspension. When dry, they were placed in cages that exposed only the area on which the eggs were laid (Munger 1957). This area was enclosed with a ventilated cover to confine the mites that hatched.

The cages were held in the rearing room, and as the mites matured they were counted and removed from the cage. The difference between the number of eggs laid and the number of mites that matured represented the mortality caused by the treatment.

The initial susceptibility of the ovex-resistant strain as compared with that of the nonresistant strain was determined, and dosage-mortality curves are shown in figure 6. The regression lines were calculated as in the demeton experiments. Slopes of the lines were consistent from one test to another, averaging 1.79 for the nonresistant and 3.12 for the resistant strain. The comparative dosages of nonresistant and ovex-resistant citrus red mites treated with ovex are shown in table 3.

TESTS FOR CROSS-TOLERANCE OF DEMETON- AND OVEX-RESISTANT STRAINS TO TEDION AND KELTHANE.—Since resistance of citrus red mites to demeton and ovex has eliminated the use of these materials in certain areas, the question arose as to the effect of the two most promising new materials, Tedion and Kelthane, on the resistant strains. Comparisons were therefore made to answer this question. The method for making these determinations was the same as that used in the ovex studies. Lemons

**Table 2.—Comparative LD50s of nonresistant and demeton-resistant citrus red mites treated with demeton, 1958.**

| TREATMENT DATE | LD50 (GRAMS PER LITER) | | RELATIVE POTENCY |
|---|---|---|---|
| | Nonresistant | Resistant | |
| Jan. 6 | 0.050 | 7.77 | 155±23.6 |
| Jan. 27 | .071 | 10.50 | 150±21.5 |
| Feb. 10 | .045 | 8.52 | 191±25.3 |
| Average | | | 163±13.5 |

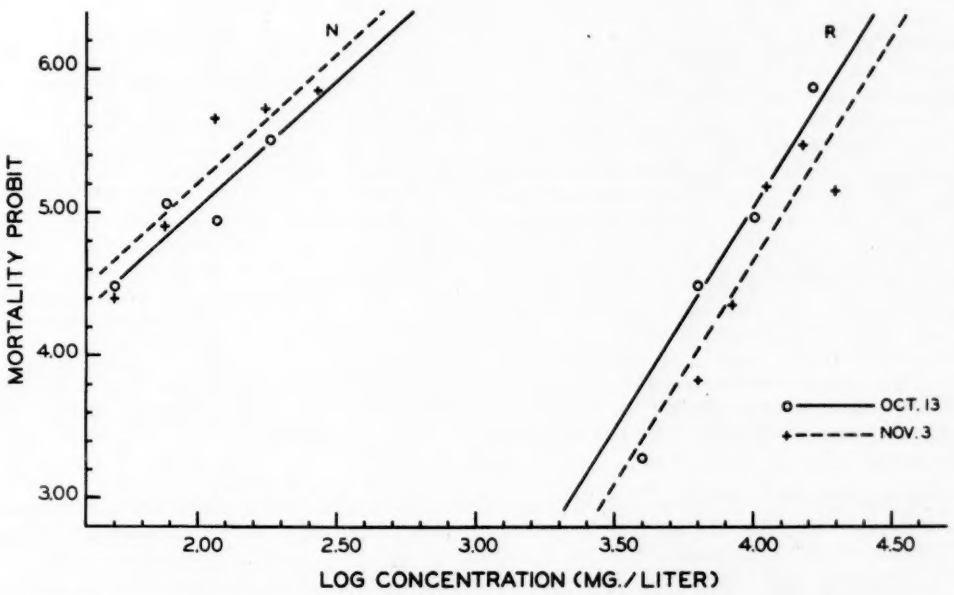


FIG. 6.—Mortality of nonresistant (N) and ovex-resistant (R) citrus red mites treated with ovex. Comparisons were made between the two strains on dates indicated.

**Table 3.—Comparative dosages of nonresistant and ovex-resistant citrus red mites treated with ovex, 1958.**

| Treatment Date | LD50 (Grams per Liter) | | LD90 (Grams per Liter) | | Relative Resistance | |
|---|---|---|---|---|---|---|
| | Nonresistant | Resistant | Nonresistant | Resistant | LD50 | LD90 |
| Oct. 13 | 0.095 | 9.55 | 0.50 | 24.55 | 100 | 49 |
| Nov. 3 | .078 | 10.23 | .40 | 32.36 | 162 | 81 |
| Average | | | | | 131 | 65 |

**Table 4.—Comparative LD50s of nonresistant, ovex-resistant, and demeton-resistant citrus red mites treated with Tedion and Kelthane.**

| Treatment Date | Strain | LD50 (Grams/Liter) | Slope of Line |
|---|---|---|---|
| | *Tedion* | | |
| Oct. 27, 1958 | Nonresistant | 0.035 | 4.58 |
| | Ovex-resistant | .034 | 3.08 |
| | Demeton-resistant | .043 | 5.52 |
| | *Kelthane* | | |
| Dec. 12, 1958 | Nonresistant | 0.355 | 4.26 |
| | Ovex-resistant | .309 | 3.64 |
| Jan. 12, 1959 | Nonresistant | .398 | 3.98 |
| | Demeton-resistant | .427 | 3.48 |

bearing eggs of the different strains were treated and placed in cages with eggs enclosed. Mortalities were based on the number of mites that matured from given numbers of eggs.

The slight differences in the slopes of the regression lines of the dosage-mortality curves did not appear to be significant. Table 4 indicates that there was no cross-tolerance to either material, since there were no significant differences in LD50s between strains. However, there was considerable difference between Kelthane and Tedion, the average LD50 for the former being 10 times that for the latter as measured by the combined mortalities of eggs and developing mites. At the dosages used, Tedion had little effect on eggs, but Kelthane had an appreciable ovicidal action which increased with dosage.

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# Field Evaluation of Sevin as an Insecticide for Pests of Vegetables in New Jersey[1,2]

Philip Granett and John P. Reed

## Abstract

Beginning in 1954 and in each subsequent year, 1-naphthyl *N*-methylcarbamate, identified either as Experimental Insecticide 7744 or Sevin®, has been tested in the field in New Jersey on a wide range of insects and vegetable crops. Using wettable powder (wp) formulations at rates of 0.25 to 1 lb., active ingredients per 100 gallons, it was found that Sevin could provide control of the cabbage flea beetle (*Phyllotreta cruciferae* (Goeze)), potato leafhopper (*Empoasca fabae* (Harr.)), European corn borer (*Pyrausta nubilalis* (Hbn.)), corn earworm (*Heliothis zea* (Boddie)), fall armyworm (*Laphygma frugiperda* (J. E. Smith)), and the Mexican bean beetle (*Epilachna varivestis* Muls.). Sevin was usually somewhat less effective at these rates against the squash bug (*Anasa tristis* (DeG.)) or cabbage leaf feeders such as the imported cabbageworm (*Pieris rapae* (L.)), cabbage looper (*Trichoplusia ni* (Hbn.)), or diamondback moth (*Plutella maculipennis* (Curt.)) larvae. It was only moderately effective against the potato aphid (*Macrosiphum solanifolii* (Ashm.)) and it appeared to favor buildup of the turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis)), presumably by destroying some parasites and predators. Sevin did not control the two-spotted mite (*Tetranychus bimaculatus* Harvey) on beans.

Foliage injury was not apparent except after repeated spraying of corn (Iochief) in 1956 and 1957. This injury was not noticed in 1958.

In general, Sevin did not appear to cause any marked effect on yield. This result was also true in respect to flavor or taste acceptability as judged by tests on crop samples taken from treated plots of lima beans, turnips, potatoes, and tomatoes. Residue analyses made on samples of crops taken on the day sprayed indicated varying amounts of active chemical depending on the crop. Broccoli retained highest amounts, but available data showed that on this and other crops Sevin residue decreased to a relatively low level within a week.

The first published summary of the insecticidal properties and characteristics of Sevin® (1-naphthyl *N*-methylcarbamate) appeared in 1957 (Haynes *et al.*). Since that time many investigators have tested this chemical on a wide range of pests and localities. Some of the information bearing specifically on its effectiveness against pests of vegetables has been presented in papers by Bottger *et al.* (1958), Davis *et al.* (1958), Graham & Krestensen (1957),

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[2] Investigations conducted under research grants sponsored by Union Carbide Chemicals Company at Rutgers University.

Hofmaster (1959), Wolfenbarger & Hibbs (1958), and Young & Ditman (1959).

Sevin was first tested in the field in New Jersey in 1954 under its identification as Experimental Insecticide 7744, an aryl urethane. The results of numerous small- and large-plot trials on vegetables during subsequent years have confirmed the fact that this insecticide is effective against a substantial number of important pests. It has a moderate residual life, is not generally injurious to plants, does not appear to affect flavor adversely, and is of relatively low mammalian toxicity. Considerable more work remains to be done to ascertain the effectiveness of Sevin against pests other than those already tried. In this survey, the data accumulated during the past 6 years have been assembled to indicate the extent of the work that has been done in New Jersey and to serve as a guide for future work.

PROCEDURES.—Most of the small-plot work has been conducted at the New Jersey Agricultural Experiment Station vegetable farm, near New Brunswick. Although the size and variety of the vegetable plantings was governed by the needs of each season, a list of the plants, types, and general plot size (replicated two to five times) follows:

| CROP | VARIETY | PLOT SIZE PER REPLICATE |
|---|---|---|
| Lima bean | Fordhook | 4 to 8 rows 12′–15′ |
| Snap bean | Black Valentine | 4 to 8 rows 12′ |
| Broccoli | Waltham | 25 to 30 plants |
| Cucumber | Marketeer | 4 to 12 plants |
| Cabbage | Dutch Bald | 25 to 30 plants |
| Squash | Cocozelle | 6 to 10 plants |
| Tomato | Rutgers | 10 to 20 plants |
| Turnip | White Purple Top | 2 to 4 rows 12′ |
| Carrots | Chantenay | 2 to 4 rows 12′ |
| Corn | Iochief | 4 to 8 rows 12′–20′ |
| Potatoes | Katahdin | 4 rows 60′ long |

Treatments were normally laid out in a randomized pattern, with untreated plots and commercially available insecticides included for comparison. Counts or observations of insects usually were made before and after treatment, control being judged by comparison with the number of living or dead insects found on or under the untreated plants. A 5-gallon knapsack sprayer was used for application of spray treatments. Sevin formulations used included wettable powders (wp) containing 25%, 50%, and 85% of the active ingredient, 24% emulsion concentrate, and 5% granulated dust. In 1955 the emulsion treatment caused some foliage injury to corn leaves. This condition was attributed to the emulsifier-solvent combination contained in this experimental concentrate. Since the emulsion concentrate formulation has not been commercially available, the results obtained with it are not included in this survey.

*Additional Tests.*—In 1956, 1957, and 1958 Sevin was included in cooperative tests conducted by the Rutgers Plant Pathology and Entomology Departments for control of insects and diseases on tomatoes and potatoes. Located on a commercial farm (Simonson Bros., Cranbury, New Jersey) the potato plots, 4 rows 60 feet long, were replicated four times and the tomato plots, 10 plants each, also were replicated four times. Seven applications were made on a regular spray schedule of 6- to 12-day intervals during the season with a power sprayer, using 250 pounds pressure and 125 to 150 gallons per acre. Evaluation of a treatment was judged by such criteria as insect counts, appearance of plants, yields obtained, effect on flavor, and comparison with standard recommended materials.

RESULTS.—*Small Plot work.*—Plants were treated for insect control as one or more pests became abundant during the seasons. A list of the common and scientific names of the various insects encountered follows:

| | |
|---|---|
| Cabbage flea beetle | *Phyllotreta cruciferae* (Goeze) |
| Striped flea beetle | *Phyllotreta striolata* (F.) |
| Imported cabbageworm | *Pieris rapae* (L.) |
| Cabbage looper | *Trichoplusia ni* (Hbn.) |
| Diamondback moth | *Plutella maculipennis* (Curt.) |
| Potato leafhopper | *Empoasca fabae* (Harr.) |
| European corn borer | *Pyrausta nubilalis* (Hbn.) |
| Corn earworm | *Heliothis zea* (Boddie) |
| Corn sap beetle | *Carpophilus dimidiatus* (F.) |
| Fall armyworm | *Laphygma frugiperda* (J. E. Smith) |
| Squash bug | *Anasa tristis* (DeG.) |
| Potato Aphids | *Macrosiphum solanifolii* (Ashm.) |
| Turnip aphid | *Rhopalosiphum pseudobrassicae* (Davis) |
| Cabbage aphid | *Brevicoryne brassicae* (L.) |
| Green peach aphid | *Myzus persicae* (Sulz.) |
| Mexican bean beetle | *Epilachna varivestis* Muls. |
| Two-spotted mite | *Tetranychus bimaculatus* Harvey |

A tabular condensation of typical results obtained from 1954 through 1957 against these insects, using Sevin and comparison insecticides, is presented in table 1. Concentrations or dosages indicated are for pounds of active ingredient per 100 gallons water. Per cent control provided by treatments was based on comparison with the number of insects found on untreated plants at the time period indicated. A measured portion of each replicate or a definite number of plants was examined for this purpose.

*Cabbage Flea Beetles.*—Effective control was obtained up to about a week after spray applications from concentrations of 0.25 to 1 lb. active Sevin. No phytotoxicity to broccoli, cabbage, or turnips was noted at these concentrations.

*Potato Leafhoppers.*—Good control was obtained at 0.5 to 1 lb., against this insect on snap beans and corn up to 2 weeks following treatments.

*Mexican Bean Beetle Larvae.*—Sevin is exceptionally effective against this pest, concentrations as low as 0.25 lb. providing almost complete kill. Excellent control has been recorded by other investigators (Brett & Brubaker 1958, and Sleesman 1957), often under conditions of greater insect populations.

*Aphids.*—Only moderate success has been obtained against several species of aphids on tomatoes and relatively poor activity against these pests on turnips, broccoli and eggplants. Tests in 1958, mentioned later, indicate that repeated applications of Sevin may favor build-up of turnip and cabbage aphids on turnips and eggplants. Young & Ditman (1959) found Sevin effective against the melon aphid (*Aphis gossypii* Glov.) but not against the potato aphid.

*Cabbage-Leaf-Feeding Insects.*—Although good control was obtained against the imported cabbageworm in one series of tests in 1956, when results in which the effectiveness of Sevin against larvae of imported cabbageworm, cabbage looper, and diamondback moth where evaluated control was found to be only moderate. It might be pointed out that in this series of tests DDT was ineffective also.

*Insects on Corn.*—These tests were made in 1954, 1955, 1956, and 1957, with plants (Iochief) being treated four to seven times during the period when European corn

**Table 1.—Results with Sevin and other insecticides compared on replicated small plots at New Brunswick, New Jersey.**

| Year and Month | Pest and Crop | Per Cent Sevin[a] Treatment | Pounds Active Ingredient/A. | Days Observed | No. of Insects on Untreated | Control (%) | Comparison Treatment[a] | Pounds Active Ingredient/A. | Control (%) |
|---|---|---|---|---|---|---|---|---|---|
| 8/54 | *Flea beetles* on cabbage | 25 | 0.25 | 1 | 162 | 88 | DDT, 50 | 1.0 | 92 |
| | | | | 2 | 60 | 97 | | | 95 |
| | | | 0.5 | 1 | 133 | 100 | | 1.0 | 98 |
| 7/56 | | 50 | 1.0 | 1 | 381 | 99 | DDT, 50 | 1.0 | 98 |
| | | | | 4 | 351 | 98 | | | 91 |
| | | | | 11 | 1,018 | 98 | | | 98 |
| | | | | 14 | 994 | 0 | | | 0 |
| 8/56 | | 50 | 0.5 | 1 | 1,430 | 98 | DDT, 50 | 0.5 | 98 |
| | | | | 8 | 968 | 55 | | | 91 |
| | | | | 15 | 977 | 46 | | | 46 |
| 7/56 | on turnips | 50 | 0.5 | 1 | 334 | 100 | DDT, 50 | 1.0 | 100 |
| | | 50 | 1.0 | 1 | 510 | 98 | DDT, 50 | 1.0 | 94 |
| | | | | 4 | 427 | 94 | | | 86 |
| | | | | 11 | 2,748 | 43 | | | 15 |
| 8/56 | | 50 | 1.0 | 1 | 1,015 | 99 | DDT, 50 | 1.0 | 97 |
| | | | | 7 | 1,693 | 55 | | | 51 |
| 7/57 | | 50 | 0.75 | 1 | 356 | 98 | DDT, 50 | 0.75 | 99 |
| | | | | 5 | | 78 | | | 90 |
| | | | | 8 | | 0 | | | 42 |
| 8/57 | | 50 | 0.5 | 1 | 484 | 98 | DDT, 50 | 0.5 | 98 |
| | | | | 5 | | 98 | | | 94 |
| | | | | 8 | | 3 | | | 0 |
| 7/57 | on broccoli | 50 | 0.25 | 1 | 1,688 | 99 | DDT, 50 | 0.25 | 95 |
| | | | | 5 | | 92 | | | 95 |
| | | | | 8 | | 84 | | | 96 |
| | | | 0.5 | 1 | 1,840 | 99 | | 0.5 | 99 |
| | | | | 5 | | 99 | | | 99 |
| | | | | 8 | | 75 | | | 51 |
| | | | 0.75 | 1 | 1,688 | 99 | | 0.75 | 99 |
| | | | | 5 | | 99 | | | 98 |
| | | | | 8 | | 89 | | | 98 |
| 6/56 | *Aphids* on tomatoes | 50 | 0.25 | 1 | 459 | 63 | Malathion, 25 | 0.25 | 91 |
| | | | | 2 | 511 | 51 | | | 85 |
| | | | | 5 | 489 | 0 | | | 84 |
| | | | 1.0 | 1 | 459 | 77 | | 0.5 | 99 |
| | | | | 2 | 511 | 74 | | | 97 |
| | | | | 5 | 489 | 64 | | | 97 |
| 7/56 | on turnips | 50 | 1.0 | 8 | Moderate | Poor | DDT, 50 | 1.0 | Moderate |
| 9/57 | on eggplant | 50 | 1.0 | 2 | 193 | 58 | Malathion, 25 | 1.0 | 98 |
| | | | | 8 | 1,090 | 71 | | | 91 |
| | | 50 | 1.0 | 1 | | 77 | Malathion, 25 | 1.0 | 92 |
| | | | | 6 | | 92 | | | 96 |
| 9/57 | on broccoli | 50 | 1.0 | 1 | Heavy | Poor | Malathion, 25 | 1.0 | Good |
| 8/56 | *Squash bug* | 50 | 1.0 | 1 | 24 | 83 | Parathion, 15 | 0.15 | 71 |
| 8/56 | *Mexican bean beetle* on snap beans | 50 | 0.25 | 1 | 272 | 98 | Malathion, 25 | 0.5 | 96 |
| | | | 0.5 | 1 | 272 | 98 | | | |
| | | | 1.0 | 1 | 259 | 97 | DDT, 50 | 1.0 | 48 |
| 9/56 | | 50 | 1.0 | 1 | 42 | 100 | Parathion, 15 | 0.15 | 50 |
| | | | | 5 | 54 | 100 | | | 98 |

[a] Wettable powder unless indicated otherwise.

**Table 1.—(*Contined*)**

| Year and Month | Pest and Crop | Per Cent Sevin[a] Treatment | Pounds Active Ingredient/A. | Days Observed | No. of Insects on Untreated | Control (%) | Comparison Treatment[a] (%) | Pounds Active Ingredient/A. | Control (%) |
|---|---|---|---|---|---|---|---|---|---|
| 8/54 | *Potato leafhopper* on lima beans | 25 | 0.5 | 1 | 182 | 96 | | | |
| 7/56 | | 50 | 1.0 | 1 | 18 | 100 | DDT, 50 | 1.0 | 72 |
| | | | | 7 | 16 | 56 | | | 56 |
| | | | | 14 | 41 | 71 | | | 78 |
| 7/57 | | 50 | 0.5 | 1 | 63 | 100 | DDT, 50 | 0.5 | 75 |
| | | | | 5 | | 96 | | | 83 |
| | | | | 8 | | 98 | | | 98 |
| | | | | 14 | | 85 | | | 95 |
| 7/57 | on corn | 50 | 0.5 | 1 | 71 | 98 | DDT, 50 | 0.5 | 76 |
| | | | | 5 | | 100 | | | 87 |
| | | | | 8 | | 96 | | | 97 |
| | | | | 14 | | 89 | | | 98 |
| 7/56 | *Imported cabbage-worm* on cabbage | 50 | 1.0 | 1 | 153 | 85 | DDT, 50 | 1 | 79 |
| | | | | 4 | 213 | 97 | | | 93 |
| | | | | 11 | 177 | 97 | | | 92 |
| 7/56 | *Cabbage leaf feeders* on cabbage[b] | 50 | 1.0 | 1 | 153 | 56 | DDT, 50 | 1.0 | 60 |
| | | | | 4 | 213 | 91 | | | 81 |
| 8/56 | on cabbage[c] | 50 | 1.0 | 1 | 98 | 60 | DDT, 50 | 1.0 | 51 |
| | | | | 9 | 70 | 54 | | | 9 |
| | | 50 | 1.0 | 2 | 75 | 71 | DDT, 50 | 1.0 | 29 |
| 8/56 | *Two-spotted mite* on snap bean | 50 | 1.0 | 1 | 329 | 0 | Malathion, 25 | 1.0 | 95 |
| | | | | 7 | 805 | 0 | | | 88 |
| | | 50 | 1.0 | 2 | 407 | 0 | Malathion, 25 | 1.0 | 78 |
| | | | | 8 | 1,194 | 0 | | | 75 |
| | | | | 1 | | 21 | Malathion, 25 | 1.0 | 76 |
| | | | | 6 | | 0 | | | 87 |
| 8/54 | *European corn borer* in ears and stalks | 25 4 times @ 5–6 day intervals | 0.5 | — | 28/100 plants | 79 | Malathion (E.C.), 57 | 0.62 | 72 |
| 9/54 | | do | 0.5 | — | 70 | 83 | Malathion (E.C.), 57 | 0.62 | 98 |
| 9/55 | | 50 4 times @ irregular intervals | 0.5 | — | 404 | 75 | DDT, 50 | 1 | 90 |
| 9/55 | | 50 7 times @ 6 day intervals | 0.5 | — | 106 | 74 | DDT, 50 | 1 | 90 |
| 10/56 | | 50 5 times @ 5–6 day intervals | 1.0 | — | 418 | 92 | DDT, 50 | 1 | 98 |
| 10/57 | | 50 6 times @ 4–7 day intervals | 1.0 | — | 90 | 97 | DDT, 50 | 1 | 99 |
| 9/54 | *Fall armyworm* on corn | 25 4 times | 0.5 | — | 92 | 100 | Malathion, 25 | 0.5 | 100 |
| 10/56 | | 50 5 times @ 5–6 day intervals | 1.0 | — | 60 | 100 | DDT, 50 | 1.0 | 100 |

[b] Imported cabbage worm and diamondback moth.
[c] Imported cabbage worm, cabbage looper, and diamond back moth.

**Table 1.—(*Continued*)**

| Year and Month | Pest and Crop | Per Cent Sevin[a] Treatment | Pounds Active Ingredient/A. | Days Observed | No. of Insects on Untreated | Control (%) | Comparison Treatment[a] (%) | Pounds Active Ingredient/A. | Control (%) |
|---|---|---|---|---|---|---|---|---|---|
| 9/55 | *Sap beet'e larvae* on corn | 50 4 times @ irregular intervals | 0.5 | — | 782 | 71 | DDT, 50 | 1.0 | 51 |
| 8/54 | *Corn earworm* on corn | 25 4 times @ 5–6 day intervals | 0.5 | — | 338 | 75 | Malathion (E.C.), 57 | 0.62 | 49 |
| 9/54 | | do | 0.5 | — | 316 | 61 | Malathion (E.C.), 57 | 0.62 | 20 |
| 9/55 | | 50 7 times @ 6 day intervals | 0.5 | — | 370 | 80 | DDT, 50 | 1.0 | 7 |
| 10/56 | | 50 5 times @ 5–6 day intervals | 1.0 | — | 194 | 83 | DDT, 50 | 1.0 | 92 |
| 10/57 | | 50 6 times @ 4–7 day intervals | 1.0 | — | 86 | 100 | DDT, 50 | 1.0 | 71 |

borer and corn earworm were active. At 0.5 and 1.0 lb. of the active ingredient it was found that Sevin was moderately effective against the European corn borer and corn earworm, and good against the fall armyworm. Some foliage injury in the form of necrotic areas and chlorosis along leaf veins was apparent from the wettable powder used in 1956 and 1957. This condition was manifest, also, by what appeared to be a possible systemic effect, the corn stalks being smaller and curved. These phytotoxic symptoms have not appeared in tests made under other conditions at other times. Eden (1958) noted, however, that when 12 applications of an experimental Sevin emulsion concentrate spray were applied, good control of corn earworm was obtained, but that severe foliage injury occurred.

*Squash Bug.*—Moderate control was obtained against this pest in one trial at a 1 pound dose.

*Two-Spotted Mite.*—Sevin was ineffective against this pest on snap beans.

*1958 Data.*—Sevin and DDT were included in a test in which the insecticides were applied periodically at approximately 2-week intervals (four to five spray applications) during the season to squash, snap beans, turnips, tomatoes, and cucumbers. Flea beetles and cabbage aphids were found in abundance on turnips; other than these pests, not enough insects occurred to make significant comparisons of control. Yield data were obtained, however, from all the crops. The information is summarized in table 2.

Two species of flea beetles, the striped and the cabbage were prevalent. As one means of judging the seasonal effectiveness of the insecticides against these insects, 13 counts were made at periodic intervals and per cent control determined by comparison with similar counts on untreated check plots. On this basis, fair to good control of these species of flea beetles was obtained from Sevin (72% at 0.25 lb. rate, 76% at 1 lb.) and DDT (68% at 0.25 lb. rate, 65% at 1 lb.), although more frequent spraying than the 2-week interval used might have been desirable when these insects were most troublesome.

After four sprays had been applied it was noticed that although predators (Coccinellidae) and parasitized turnip aphids were observed on the untreated turnips and, to a lesser extent, on the DDT-treated plots, aphids were present in large numbers on turnips treated with Sevin. Despite an extra spray treatment, control of the aphids on these plots was not achieved and the plants were severely

**Table 2.—Yields from plants treated with wettable-powder sprays four times during the 1958 season.[a]**

| Plants Treated | Pounds Active Ingredient/A. | Yields (Lb.) Sevin | DDT | Untreated |
|---|---|---|---|---|
| | | *Per Plant* | | |
| Squash | 0.25 | 11.6 | 10.3 | |
| | 1.0 | 14.8 | 9.7 | |
| | | | | 14.5 |
| | | *From 9′ Row Replicated 3 Times* | | |
| Snap beans | 0.25 | 14.8 | 18.0 | |
| | 1.0 | 15.8 | 18.0 | |
| | | | | 16.3 |
| | | *From 9′ Row Replicated 3 Times* | | |
| Turnips | 0.25 | 19.2 Turnips | 29.2 Turnips | |
| | | 24.8 Tops | 33.2 Tops | |
| | 1.0 | 12.2 Turnips | 32.2 Turnips | |
| | | 16.0 Tops | 39.5 Tops | |
| | | | | 27.0 Turnips |
| | | | | 28.3 Tops |
| | | *From 10 Plants Replicated 3 Times* | | |
| Tomatoes | 0.25 | 87.5 | 84.4 | |
| | 1.0 | 112.4 | 109.2 | |
| | | | | 80.1 |
| | | *Per Plant* | | |
| Cucumbers | 0.25 | 30.2 | 34.3 | |
| | 1.0 | 33.3 | 25.9 | |
| | | | | 27.4 |

[a] Four spray treatments at 10-day to 2-week intervals beginning July 7, 1958. One extra spray application on turnips. No active insect trouble occurred except for flea beetles and aphids on turnips.

**Table 3.—Total number of insects collected from insecticide-treated potato plots on Simonson Bros. Farm, Cranbury, New Jersey.[a]**

| Year | Treatment (WP) | Pounds Active per 100 Gal. $H_2O$ | Potato Aphid | Flea Beetle | Leafhoppers | | Lygus Bugs | Corn Borer (60 plants) |
|---|---|---|---|---|---|---|---|---|
| | | | | | Potato | Others | | |
| 1956 | Sevin | 1.0 | 616 | 84 | 46 | 63 | 9 | 41 |
| | Methoxychlor | 1.5 | 1,117 | 179 | 37 | 53 | 23 | 36 |
| | DDT | 1.0 | 1,009 | 149 | 39 | 82 | 25 | 37 |
| | Endrin | 0.5 | 274 | 55 | 57 | 136 | 24 | 35 |
| 1957 | Sevin | 1.0 | 1,208 | 5 | 34 | 55 | 4 | |
| | DDT | 1.0 | 1,708 | 3 | 47 | 71 | 6 | |
| | Dieldrin | 0.5 | 411 | 5 | 89 | 128 | 9 | |
| | DDT+parathion | 1.0 | | | | | | |
| | | 0.15 | 285 | 8 | 28 | 66 | 6 | |
| | Thiodan®[b] | 0.5 | 131 | 8 | 78 | 115 | 8 | |
| 1958 | Sevin | 1.0 | 285 (912) | 10 | 10 | 28 | 20 | |
| | DDT | 1.0 | 1,025 (732) | 20 | 12 | 26 | 8 | |
| | Dieldrin | 0.5 | 769 (490) | 14 | 15 | 42 | 13 | |
| | Endrin | 0.375 | 147 (211) | 12 | 15 | 19 | 6 | |
| | Thiodan | 0.5 | 172 (269) | 3 | 16 | 31 | 17 | |

[a] 40 net sweeps on each collecting date (15 in 1956, 14 in 1957, and 13 in 1958). Figures in parenthesis for 1958 aphid counts are green peach aphid.
[b] 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide.

**Table 4.—Quality and yield data obtained from samples of several crops.[a]**

| Year | Crop | Treatment[b] | Pounds Active per 100 Gal. | Yield per Acre | Quality | |
|---|---|---|---|---|---|---|
| | | | | | Flavor | Acceptability |
| 1955 | Lima beans | Sevin | 0.5 | — | Similar | 100 |
| | | DDT | 1.0 | — | Similar | 100 |
| | Turnips | Sevin | 0.5 | — | Similar | 100 |
| | | DDT | 1.0 | — | Similar | 100 |
| 1956 | Tomatoes | Sevin | 1.0 | 19.9 tons | Different | 87 |
| | | Parathion, e.c. | 0.25 | 20.9 | Similar | 91 |
| | | Dieldrin | 0.5 | 23.5 | Different | 91 |
| | | TDE | 1.0 | 21.8 | Similar | 100 |
| | Potatoes | Sevin | 1.0 | 552 bu. | Similar | 94 |
| | | Methoxychlor | 1.5 | 531 | Different | 100 |
| | | DDT | 1.0 | 528 | Different | 94 |
| | | Endrin | 0.5 | 618 | Different | 94 |
| 1957 | Tomatoes | Sevin | 1.0 | 17.0 tons | Similar | 100 |
| | | Parathion, ec | 0.25 | 22.1 | Different | 94 |
| | | Dieldrin | 0.5 | 19.2 | Similar | 100 |
| | | TDE | 1.0 | 17.5 | Similar | 100 |
| | Potatoes | Sevin | 1.0 | 415 bu. | Similar | 97 |
| | | DDT | 1.0 | 425 | — | 100 |
| | | Dieldrin | 0.5 | 478 | Similar | 93 |
| | | DDT+parathion | 1.0 | | | |
| | | | 0.15 | 440 | Different | 93 |
| | | Thiodan | 0.5 | 471 | Different | 87 |
| 1958 | Tomatoes | Sevin | 1.0 | 11.8 tons | Different | Comparable |
| | | Parathion, ec | 0.25 | 11.9 | Different | Comparable |
| | | Dieldrin | 0.5 | 12.2 | Different | Sl. Poorer |
| | | TDE | 1.0 | 12.4 | Different | Sl. Poorer |
| | Potatoes | Sevin | 1.0 | 483 bu. | Different | Comparable |
| | | DDT (Coded check) | 1.0 | 520 | — | Comparable |
| | | Dieldrin | 0.5 | 535 | Different | Poorer |
| | | Endrin | 0.375 | 531 | Different | Sl. Poorer |
| | | Thiodan | 0.5 | 521 | Different | Sl. Poorer |

[a] Lima beans and turnips sprayed twice prior to sampling. Tomatoes and potatoes sprayed seven times during season.
[b] Wettable powders unless indicated otherwise.

**Table 5.—Residues of Sevin on various vegetable crops grown in New Jersey and elsewhere.[a]**

| Crop | Days After Spray | Average Residue (P.P.M.) | Sensitivity of Method (P.P.M.) |
|---|---|---|---|
| Tomatoes | 0 | 1.62 | 0.06 |
| | ( 0) | (2.65) | |
| | ( 1) | (2.14) | |
| | ( 3) | (1.47) | |
| | ( 7) | (1.01) | |
| | (14) | (0.28) | |
| Potatoes | 33 | 0.03 | .05 |
| Cucumbers | 0 | .11 | .06 |
| | ( 0) | (3.64) | |
| | ( 1) | (1.68) | |
| | ( 3) | (1.21) | |
| | ( 7) | (0.75) | |
| Broccoli | 0 | 13.1 | .10 |
| | ( 0) | (8.62) | |
| | ( 1) | (3.18) | |
| | ( 8) | (1.99) | |
| | ( 7) | (0.67) | |
| Snap beans | 0 | 0.03 | .11 |
| | 3 | .03 | |
| | ( 0) | (1.5 ) | |
| | ( 1) | (0.8 ) | |
| | ( 3) | (0.3 ) | |

[a] Applied at the rate of 1 lb. active ingredient per acre.
Analyses made by quality control laboratory of Union Carbide Chemicals Co. Figures in parenthesis are maximum averages obtained from samples of crops sent in from other localities combined with those from New Jersey.

damaged. This may account for the low yield obtained from the Sevin treatments when the turnips were finally harvested.

In the absence of major insect damage on most of the crops, yield differences between treated or untreated plants was not marked except on tomatoes where Sevin-treated plants showed an increase and on turnips, as already noted. On squash and beans, yields from treated plots did not appear to be significantly different from those obtained from untreated plants.

*Additional Tests on Potatoes and Tomatoes.*—Observations of insect abundance, yield data, and information on quality of the crop were included as part of the cooperative testing of insecticides conducted by the Plant Pathology and Entomology Departments during 1956, 1957, and 1958. A summary of the results of net sweepings from potato plots sprayed with Sevin and other insecticides is presented in table 3.

It was found that Sevin was only moderately effective or weak against aphids, and usually good against flea beetles, leafhoppers, and lygus bugs. In 1956 the number of corn borers found in the Sevin plots did not appear to be markedly different from the number in other insecticide treatments.

No buildup of the insects commonly found on tomatoes occurred in 1956, 1957, or 1958. During the 3 years, however, cabbage looper damage was found to be moderate to severe on the Sevin and TDE plots; in 1957 two-spotted mites were abundant in the check and Sevin treatments.

*Quality Data.*—Samples of vegetables, potato tubers, and tomato fruits were submitted to the Rutgers Food Science Department for flavor and acceptability evaluations. In these tests, flavor was expressed in terms of similarity to or difference from a check sample. A flavor evaluation of *similar* means that the panel score or rating of the treated sample *does not* differ from the rating of the check sample by more than the least significant difference between panel scores for all the samples that were tested. When the treated samples were rated as *different*, such samples *differed* from the check samples by more than the least significant differences between panel scores.

The panels consisted of 9 to 12 tasters who judge flavor and express their personal opinions on the acceptability of each sample. It is emphasized, however, that such a panel rating of acceptability does not necessarily mean consumer preference.

Flavor and acceptability ratings on treated lima beans and turnips submitted in 1955 and on tomatoes and potatoes treated in the cooperative tests are presented in table 4. Yield records for the last-mentioned two crops are included in the tabulation.

*Residues.*—Residue analyses were made by the quality control laboratories of Union Carbide Chemicals Company at South Charleston, West Virginia. These analyses have been obtained from samples of tomatoes, potatoes, cucumbers, broccoli, and snap beans sprayed with wettable powder formulations of Sevin in New Jersey. The average residues in parts per million of active chemical (1-naphthyl-*N*-methylcarbamate) for the samples of these crops and, where the information was available, the maximum average for the same crops grown in other localities, are shown in table 5. These data indicate that although residues found on the samples taken on the same day of spraying varied for each crop, the amount became progressively less on succeeding days and was relatively low at the end of 1 week. Retention of Sevin was apparently highest in such a crop as broccoli, but even in broccoli Sevin had declined from an average of 8.62 p.p.m. to 0.67 p.p.m. 7 days after treatment.

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# The Effect of Copper Compounds on Control of Citrus Rust Mite with Zineb[1]

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ABSTRACT

Zineb (zinc ethylene-bis-dithiocarbamate) was tested with six copper compounds and with basic copper sulfate plus emulsified oil or parathion against citrus rust mite, *Phyllocoptruta oleivora* (Ashm.). The dosage of 0.5 lb. 65% zineb per 100 gallons of water was far more effective than 10.0 lb. sulfur. All the copper compounds reduced the effectiveness of zineb in summer sprays, but not in postbloom sprays. One pound of 65% zineb plus copper was superior to sulfur although less effective than zineb alone. Emulsified oil, but not parathion, improved control with zineb in sprays containing copper.

Copper compounds are used on Florida citrus to control scab, melanose, and greasy spot. These diseases must be controlled when citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), is or is about to become a problem. Consequently, it is often necessary to include a material for control of citrus rust mite and a copper compound in the same spray.

Preliminary experiments (Johnson *et al.* 1957) showed that although zineb (zinc ethylene-bis-dithiocarbamate) controls citrus rust mite far more effectively than sulfur, the interval of control is shortened when zineb and basic copper sulfate are used in the same spray. Since the chief advantage of zineb over sulfur is the longer period of control and consequent reduction in the number of sprays per year, the concurrent use of a copper compound could largely eliminate the value of zineb.

In the preliminary work, however, only one copper compound was used in a single application. This spray was applied May 31, 1957, and was properly timed to control citrus rust mite, but was too late to control either scab or melanose and too early to control greasy spot. The proper time to apply sprays for combined control of citrus rust time, scab, and melanose is immediately after bloom; whereas July is best for combined control of rust mite and greasy spot. Thus, the purpose of the experiments reported here was to determine: (1) whether all the copper compounds commonly used on Florida citrus reduce the effectiveness of zineb against citrus rust mite; (2) whether copper compounds reduce the effectiveness of zineb in properly timed postbloom and summer sprays; (3) whether there is any advantage to zineb-copper sprays over sulfur; and (4) whether the scalecides parathion and emulsified oil affect control of citrus rust mite when included in zineb–copper sprays.

MATERIALS AND METHODS.—Two experiments were conducted in citrus groves near Lake Alfred, Florida, during 1958. Sprays in both tests were applied immediately after bloom and again in July. Sprays were applied with a conventional hydraulic sprayer equipped with two double Boyce guns and operated at approximately 600 p.s.i. at the pump. Applications were made when there was little air movement and were brushing-type sprays applied to the point of runoff with no effort made to secure coverage of inside limbs and foliage.

Treatments were evaluated for effectiveness against citrus rust mite by frequent determinations of the density of mite populations and by a harvest examination of fruit for rust mite damage. The density of rust mite populations on fruit was determined for each plot by examination of 25 fruits per tree in the following manner: one lens field on the unexposed surface and a similar area on the exposed surface were examined with a 10X hand lens. Fruits were considered infested if one or more mites occurred in a lens field. The extent of the rust mite injury, called russet, was determined at the approximate harvest date for each variety. This determination was made by four persons who each examined 25 fruits per tree.

In experiment 1, test trees were the variety Pineapple. Plots consisted of four replications of one tree randomized in blocks. Materials and dosages are shown in table 1. The dosage of each copper compound provided 0.75 lb. of metallic copper per 100 gallons of spray. Standard treatments were sulfur and two dosages of zineb; unsprayed check plots were also employed.

In addition to determining the number of fruits infested with rust mite, mites were counted in three lens fields on each of 25 tagged fruit per plot in five treatments.

In experiment 2, test trees were the variety Valencia. Experimental plots, which consisted of one tree, were in randomized blocks with six replications. Materials and dosages are shown in table 3. Standard treatments were sulfur and two amounts of zineb. Check plots were also available.

RESULTS.—*Experiment 1:*—Comparison of the number of fruit infested with rust mite with the number of mites per lens field showed a similarity between the two sets of data. Both methods of assaying the density of rust mite populations are therefore of similar value.

The postbloom spray was applied when sampling showed no rust mite on either young fruit or foliage. Under these conditions, all treatments were preventative and

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**Table 1.—Comparison of sulfur or zineb applied April 21 and July 31, 1958 with mixtures of zineb and copper compounds against citrus rust mite.**

| Materials and Pounds per 100 Gallons | Mean Number of Infested Fruit per 25 Fruit on Indicated Dates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 6/12 | 7/7 | 7/25 | 8/19 | 9/9 | 10/1 | 10/20 | 11/12 | 12/10 |
| Unsprayed | 2.8 | 21.3 | 25.0 | 16.0 | 2.0 | 2.0 | 0.8 | 1.5 | 0.0 |
| Wettable sulfur, 10 | 0.8 | 12.0 | 20.0 | 1.0 | 6.0 | 12.5 | 14.0 | 13.0 | 8.3 |
| 65% zineb, 0.5 | 0.0 | 6.3 | 17.5 | 1.5 | 0.3 | 1.0 | 0.5 | 2.3 | 4.5 |
| 65% zineb, 1.0 | 0.0 | 6.8 | 11.0 | 0.5 | 0.8 | 0.3 | 2.3 | 0.0 | 0.0 |
| 65% zineb, 0.5+basic copper sulfate,[a] 1.4 | 0.5 | 6.3 | 19.3 | 12.8 | 13.5 | 17.3 | 8.0 | 5.8 | 3.8 |
| 65% zineb, 1.0+basic copper sulfate,[a] 1.4 | 0.3 | 4.8 | 19.8 | 4.3 | 3.8 | 6.8 | 4.5 | 4.3 | 4.3 |
| 65% zineb, 0.5+copper oxide,[b] 1.0 | 0.5 | 7.5 | 18.8 | 14.8 | 16.5 | 15.3 | 9.3 | 5.3 | 2.3 |
| 65% zineb, 0.5+copper hydroxysulfate,[c] 5.8 | 1.5 | 7.5 | 22.8 | 20.0 | 21.8 | 18.3 | 8.5 | 5.0 | 2.5 |
| 65% zineb, 0.5 +copper sulfate, 3.0+lime, 1.2 | 4.5 | 10.5 | 17.5 | 22.0 | 19.8 | 20.8 | 10.3 | 5.5 | 2.3 |
| 65% zineb, 0.5+tetra copper calcium oxychloride,[d] 1.7 | 0.3 | 5.5 | 15.8 | 14.5 | 19.0 | 18.3 | 6.2 | 5.0 | 1.5 |
| 65% zineb, 0.5+zinc sulfate,[e] 3.0+lime, 1.0 | 0.0 | 4.0 | 13.3 | 2.8 | 3.3 | 7.0 | 9.0 | 13.5 | 9.8 |
| L.S.D. at 5% level | 0.9 | 2.9 | n.s. | 3.3 | 3.4 | 3.8 | 3.7 | 3.7 | 1.2 |
| at 1% level | 1.2 | 3.9 | n.s. | 4.4 | 4.6 | 5.1 | 5.0 | 4.9 | n.s. |

[a] 53% metallic copper.
[b] 75% metallic copper.
[c] 13% metallic copper.
[d] 45% metallic copper.
[e] 36% metallic zinc.

successfully delayed the summer increase in mite numbers. Although there was little difference among the treatments, sulfur and the mixture of 0.5 lb. 65% zineb plus copper sulfate and lime did not give as good control as zineb alone (table 1).

The summer spray was applied when mites were numerous but decreasing on unsprayed trees. Under these conditions, 0.5 lb. 65% zineb gave excellent control except when used with copper compounds. Not only did the mixtures of copper compounds with 0.5 lb. zineb fail to bring mites under control, but they maintained the population at abnormally high levels through November. Sprays containing sulfur or the mixture of 0.5 lb. zineb with zinc sulfate and lime gave a fairly good initial kill, but ultimately resulted in abnormally high populations through November. The mixture of 1.0 lb. zineb with basic copper sulfate, although superior to sulfur, was not so good as zineb alone (table 1). Summary data in table 2 show: (1) prevention of fruit russet was dependent upon good control of citrus rust mite; (2) two applications of zineb at 0.5 to 1.0 lb. per 100 gallons were the only treatments that gave good control of rust mite from April 21 to December 10, 1958; (3) all the copper compounds reduced the effectiveness of 0.5 lb. zineb to the point where it was no more effective than sulfur; and (4) 1.0 lb. of zineb with a copper compound was superior to sulfur, but not quite so good as zineb alone.

*Experiment 2.*—All postbloom treatments, applied when a moderate but increasing population of rust mite was present on leaves and fruit, gave adequate control until the latter part of June. By mid-July, mites were as numerous on sulfur-sprayed trees as on check trees and significantly more numerous than in all other plots.

**Table 2.—Comparison of the average number of fruit infested with citrus rust mite, mites per lens field and russet fruit in plots sprayed with sulfur, zineb, and mixtures of zineb with copper compounds.[a]**

| Materials and Pounds per 100 Gallon | Average Number of[b] | | |
|---|---|---|---|
| | Infested Fruit[c] | Mites per Lens Field[c] | Russet Fruit[d] |
| Unsprayed | 7.1 | 3.8 | 16.5 |
| Wettable sulfur, 10 | 8.8 | 4.2 | 15.7 |
| 65% zineb, 0.5 | 3.4 | 1.2 | 2.9 |
| 65% zineb, 1.0 | 2.2 | .1 | .4 |
| 65% zineb, 0.5+basic copper sulfate, 1.4 | 8.7 | 3.1 | 16.0 |
| 65% zineb, 1.0+basic copper sulfate, 1.4 | 5.3 | | 6.7 |
| 65% zineb, 0.5+copper oxide, 1.0 | 9.0 | 3.5 | 15.4 |
| 65% zineb, 0.5+copper hydroxysulfate, 5.8 | 8.4 | | 18.2 |
| 65% zineb, 0.5+copper sulfate, 3.0+lime, 1.2 | 12.1 | | 17.4 |
| 65% zineb, 0.5+tetra copper calcium oxychloride, 1.7 | 8.0 | | 17.4 |
| 65% zineb, 0.5+zinc sulfate, 3.0+lime, 1.0 | 6.3 | | 12.9 |
| L.S.D. at 5% level | 1.6 | 1.1 | 2.6 |
| at 1% level | 2.2 | 1.6 | 3.3 |

[a] Applied April 21 and July 31, 1958.
[b] Average number in samples of 25 fruit.
[c] Average of nine mite counts made between June 12 and December 10, 1958.
[d] Fruit examined January 6 to 8, 1959.

**Table 3.—Comparison of the average number of fruit infested with citrus rust mite with russet fruit in plots sprayed with sulfur, zineb, and mixtures of zineb, basic copper sulfate, and emulsified oil or parathion. 1958.**

| Materials and Pounds per 100 Gallon[a] | Average Number of[b] | |
|---|---|---|
| | Infested Fruit[c] | Russet Fruit[d] |
| Unsprayed | 10.0 | 21.8 |
| Wettable sulfur, 10 | 6.4 | 11.2 |
| 65% zineb, 0.5 | 1.8 | 3.4 |
| 65% zineb, 0.5+basic copper sulfate,[e] 1.4 | 3.8 | 10.8 |
| 65% zineb, 0.5+0.7% oil | .8 | 1.8 |
| 65% zineb, 0.5+0.7% oil+basic copper sulfate,[e] 1.4 | 2.1 | 4.3 |
| 65% zineb, 0.5+15% parathion, 1.0 | 1.8 | 3.4 |
| 65% zineb, 0.5+15% parathion, 1.0+basic copper sulfate,[e] 1.4 | 3.9 | 9.0 |
| 65% zineb, 1.0 | 1.1 | 2.3 |
| L.S.D. at 5% level | 1.3 | 2.1 |
| at 1% level | 1.8 | 2.8 |

[a] Applied May 1 and July 22.
All plots sprayed with parathion and Tedion between September 4 and 25.
[b] Average number in samples of 25 fruit.
[c] Average of eight mite counts made between June 5 and November 14, 1958.
[d] Fruit examined December 9 and 10, 1958.
[e] 53% metallic copper.

Summer treatments were applied when the population was declining and, aided by parathion applied in September, gave satisfactory control until November. In November, however, it was apparent that basic copper sulfate had reduced the effectiveness of zineb where emulsified oil was not employed. Data summarized in table 3 show: (1) that prevention of fruit russet was dependent upon good control of citrus rust mite; (2) that sulfur was better than no treatment but significantly poorer than zineb; (3) that basic copper sulfate reduced the effectiveness of 0.5 lb. of zineb to the point where it was no more effective than sulfur; and (4) that control with zineb in sprays containing copper was improved by the inclusion of emulsified oil but not by parathion.

DISCUSSION.—All the copper compounds used on Florida citrus are considered equally effective for disease control and no satisfactory substitutes are known, but zineb is by far the most effective material for rust mite control. A reduction in the number of sprays per year thus depends upon the successful use of zineb.

In the experiments reported here, control of citrus rust mite with 0.5 lb. 65% zineb was not reduced by neutral copper in postbloom sprays. In summer sprays, however, such mixtures were of no value in one experiment, but satisfactory in the second. These differences in summer results between the two experiments are much larger than can be attributed to the September use of parathion in the second experiment. Satisfactory results were obtained with zineb–copper mixtures in both postbloom and summer sprays only where mite populations were at moderate to low levels at the time of application or where the dosage of zineb was increased to 1.0 lb. Failure occurred where mites were very numerous. Since citrus rust mite is occasionally very numerous immediately after bloom, it is believed that no less than 1.0 lb. 65% zineb should be used in postbloom sprays containing a neutral copper compound. Zineb is not recommended for summer control of citrus rust mite where copper is also required unless emulsified oil is also used in preference to parathion. Furthermore, copper sulfate and lime should not be used with zineb.

Although further information is needed, it is believed that the above recommendations will result in successful use of zineb–copper mixtures and will be an improvement over sulfur for the control of citrus rust mite in Florida.

### REFERENCES CITED

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# Field Tests with Various Insecticides for the Control of Lygus Bugs and the Corn Earworm on Lima Beans[1]

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ABSTRACT

In a series of replicated field tests in southern California against lygus bugs, principally *Lygus hesperus* (Kngt.) and the corn earworm (*Heliothis zea* (Boddie)) on lima beans, the best control of lygus bugs was given by dieldrin, toxaphene, endrin, DDT-toxaphene, and DDT (mentioned in order of their effectiveness). In reducing pitting of beans caused by lygus feeding, DDT-toxaphene was the most effective. It also gave the highest yield of dry beans (4 sacks per acre more than the check), followed by dieldrin, endrin, and DDT. Endrin was the most effective material for the combined control of lygus and the corn earworm. Toxaphene, DDT-toxaphene, and DDT alone gave adequate control of both insects and were less costly than endrin. DDT is the least expensive; one application will give good control of lygus bugs if properly applied at the right time, and a second application will reduce corn earworm damage and prevent reinfestation of lygus.

Lygus bugs, principally *Lygus hesperus* (Kngt.), have been responsible for considerable injury to lima beans in southern California since 1944. DDT has been used extensively for the control of this pest, and recently a mixture of DDT and toxaphene has been used, especially in areas where lygus resistance to DDT has developed. The corn earworm (*Heliothis zea* (Boddie)) has at times caused serious damage to lima beans. Experiments were begun at Whittier, California, in 1953 and continued through 1959 to compare various insecticides with DDT in their initial and lasting effectiveness against both these insects and in their cost. The experiments were conducted on Ventura and Fordhook lima beans and on blackeye beans in commercial fields in Orange and Los Angeles Counties.[3]

Andres *et.al.* (1955) and Hills *et al.* (1956) reported that lygus bugs have become resistant to DDT in recent years. Middlekauff & Stevenson (1952) reported good control of lygus with DDT and toxaphene on blackeye beans in Stanislaus County. Stone & Foley (1959) in experiments in southern California found that properly timed DDT applications were effective, reduced injury, and increased the yield of lima beans.

GENERAL METHODS.—When one application was scheduled, it was made at the beginning of pod formation; when there were two or three, the first was made at the peak of blossoming and the others at 2- or 3-week intervals thereafter. Dusts were applied with an 8-row power duster between 6 and 8 a.m. Emulsion sprays were applied at 13 to 35 gallons per acre with an 8-row power sprayer operating at a pressure of 125 p.s.i. and having a boom with 2 nozzles per row. To discourage the buildup of the two-spotted spider mite (*Tetranychus telarius* (L.)), the entire experimental plantings were sprayed with Aramite®

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[2] Deceased May 1959.

[3] The fields of Alderson Farms, R. E. Beem, W. T. Bushard, Clyde Casey, A. Changula, Y. K. Chikasawa, Griset Bros., and George Callens were used in these experiments.

(definitions of chemicals bearing registered trademark names are given at the end of the paper) at 1 pound per acre before the lygus treatments were made. Aramite had been found to have no insecticidal value against lygus nymphs or adults.

The effect of the treatments on lygus populations was determined weekly by making five sweeps on the bean foliage near the center of each plot with a 15-inch insect net between 8 a.m. and 1 p.m. and counting the adults and nymphs collected. Counts were made over a period of 6 to 8 weeks. When the beans were threshed, 2-pound samples from each plot were retained and 100 to 200 beans per sample examined for pitting or malformation. In some experiments, when time permitted, all pods on three to five plants per plot were picked and examined externally for injury caused by corn earworms. From 50 to 100 of these pods per plot were shelled and the beans examined for injury by lygus or corn earworms. Yield data were obtained by harvesting and weighing the beans from 1/100-acre areas in the center of each plot.

EXPERIMENTS IN 1953.—The initial tests on lygus bugs were conducted in the Changula field on Ventura limas where nine insecticides formulated as dusts were compared with a DDT dust. Plots were 12 rows wide by 150 feet long, and replicated four times. The materials were applied July 21, when lygus populations had reached one or more bugs per sweep and when blossoms and small pods were present. The beans were planted May 18 and harvested September 21.

With the exception of Aramite and sulfur, which gave no control, all the materials were responsible for significant reductions in lygus populations for 7 weeks (table 1). When judged on the basis of yield of dry beans, all material except aldrin and Aramite gave significant increases. Dieldrin and isodrin were superior, with more than 21 sacks of beans per acre.

From the standpoint of injury based upon the examination of threshed beans, while no significant difference occurred between materials, DDT with or without sulfur was the most effective, with only 1.5% to 3% of beans injured. In bean injury caused by the corn earworm, no significant difference was apparent between treatments or check.

Dusts also were tested in a late-planted field of Fordhook beans on the Chikasawa farm. Plots were 14 rows wide by 300 feet long, with four replicates. The insecticides were applied once on September 9, when small to medium-size pods were present. All but isodrin gave significant reductions in lygus populations (table 1). Heptachlor was slightly better, but all other materials were about equal in effectiveness. Apparently lygus bugs were not a serious problem, as inspection of beans in 50 green mature pods per plot showed less than 0.6% pitted beans. Corn earworms were unusually destructive, and against this insect DDT was the most effective material, with only 3% injured beans.

In an experiment where insecticides were applied for control of the two-spotted spider mite on Ventura lima beans, samples were inspected at harvest-time for lygus injury. The following dusts were applied with rotary hand dusters at 30 pounds per acre on June 11 and July 2: Ovex 5% and 10%, sulfur 100%, parathion 2%, malathion 5%, demeton 2%, Chlorobenzilate® 4%, Aramite 3%, and Aramite 3% plus sulfur 50%. Inspection of 1,200 threshed beans per treatment showed pitting ranging from 0.8% to 2% but with no significant differences between treatments and check. None of the materials showed promise against lygus bugs when used at these dosages.

EXPERIMENTS IN 1954.—Seven materials were applied as dusts against lygus on blackeye beans on the Alderson farm. Plots were 14 rows wide by 159 feet long, with five replicates. One application was made August 6, when pods were forming and when lygus populations averaged 0.7 per sweep. Data on injury were obtained by picking 150 pods from 4 or 5 plants per plot at harvest time (September 2) and examining 500 beans from each. All materials were effective for a 3-week period (table 2). Toxaphene and endrin were somewhat more effective than the other materials. Lygus populations of this size do not ordinarily cause measurable damage, and the percentage of pitted

**Table 1.—Effect of single applications of insecticide dusts against lygus bugs and corn earworms on lima beans, 1953.**

| Insecticide and Pounds per Acre | Average no. of Adults and Nymphs per Sweep: Before Treatment (July 20) | After Treatment (July 21–Sept. 7) | Per Cent of Beans Damaged by—: Lygus | Earworms | Dry Beans per Acre (100-Lb. Sacks) |
|---|---|---|---|---|---|
| *Changula Field* | | | | | |
| DDT 2.8-sulfur 28 | 1.4 | 0.23 | 1.5 | 0.2 | 18.1 |
| Toxaphene 4.0 | 1.3 | .23 | 4.5 | 2.2 | 16.4 |
| Heptachlor 0.67 | 1.0 | .25 | 8.0 | .2 | 19.2 |
| Aldrin 1.1 | 1.5 | .25 | 8.0 | 1.3 | 14.0 |
| DDT 1.8 | 1.2 | .28 | 3.0 | .2 | 16.3 |
| Dieldrin 0.6 | 1.2 | .30 | 2.0 | .9 | 21.7 |
| Chlordane 1.8 | 1.0 | .41 | 14.5 | 1.9 | 19.6 |
| Isodrin 0.7 | 1.4 | .42 | 14.0 | 0.7 | 21.6 |
| Sulfur 48 | 1.2 | .71 | 10.5 | 0 | 16.2 |
| Aramite 1.3 | 1.2 | .72 | 15.5 | .9 | 14.2 |
| Check | 1.2 | .79 | 9.5 | .8 | 12.9 |
| L.S.D. at 5% level | — | .18 | n.s.[a] | n.s.[a] | 2.0 |
| *Chikasawa Field* | | | | | |
| Heptachlor 0.7 | 1.1 | .23 | 0 | 12.7 | |
| Toxaphene 3.0 | 1.1 | .28 | 0.2 | 7.0 | |
| Dieldrin 0.3 | 0.8 | .29 | 0.1 | 7.5 | |
| DDT 1.6 | 1.1 | .31 | 0.6 | 3.1 | |
| Aldrin 0.7 | 1.0 | .36 | 0 | 7.5 | |
| Isodrin 0.5 | 0.8 | .69 | 0 | 10.2 | |
| Check | 0.6 | .76 | 0.5 | 9.9 | |
| L.S.D. at 5% level | — | .23 | n.s. | 5.3 | |

[a] Not significant according to *F* test.

**Table 2.—Effect of single applications of insecticides as dusts against lygus bugs on blackeye beans, Alderson farm, 1954.**

| Insecticide and Pounds per Acre | Numbers of Adults and Nymphs per Sweep: Before Treatment (Aug. 3) | After Treatment (Aug. 7–Sept. 1): Adults | Nymphs | Threshed Beans Pitted (%) |
|---|---|---|---|---|
| Toxaphene 2.6 | 0.8 | 0.12 | 0.0 | 0.04 |
| Endrin 0.7 | .3 | .12 | .02 | .0 |
| Dieldrin 0.5 | .4 | .17 | .0 | .04 |
| Aldrin 0.7 | 1.0 | .11 | .06 | .08 |
| Diazinon® 1.0 | .7 | .03 | .15 | .08 |
| DDT 1.5 | .9 | .17 | .04 | .0 |
| Heptachlor 1.1 | .4 | .13 | .08 | .08 |
| Check | .9 | .22 | .23 | .50 |
| L.S.D. at 5% level | — | N.S. | .12 | .03 |

Table 3.—Control of lygus bugs and corn earworms on Fordhook lima beans with three applications of insecticide dusts, Buschard field, 1954.

| Insecticide and Pounds per Acre —Application | No. of Lygus Adults and Nymphs per Sweep | | Pods Damaged by Earworms | |
|---|---|---|---|---|
| | Before Treatment (Aug. 12) | After Treatment (Aug. 13–Sept. 16) | Per Cent (By Weight) | Per Cent Control |
| Endrin 0.43 | 1.0 | 0.47 | 2.2 | 94 |
| DDT 1.4-sulfur 14 | 1.3 | .34 | 2.9 | 92 |
| Dieldrin 0.5 | 0.95 | .14 | 15.9 | 54 |
| Toxaphene 2.2 | 1.4 | .40 | 20.1 | 41 |
| Heptachlor 0.8 | 1.4 | .30 | 22.4 | 35 |
| Diazinon 0.4 | 0.7 | .49 | 23.4 | 32 |
| Isodrin 0.5 | 1.3 | .44 | 28.0 | 18 |
| Aldrin 0.6 | 1.1 | .38 | 29.2 | 15 |
| Parathion 0.5 | 1.3 | .28 | 29.1 | 15 |
| Chlordane 1.5 | 1.0 | .28 | 29.7 | 13 |
| Check | 1.3 | .78 | 34.2 | — |
| L.S.D. at 5% level | — | N.S. | 7.3 | — |

beans was low. Nevertheless, all materials gave significant reductions in damage.

In another experiment with dusts, a late-planted field of Fordhook beans was used (Buschard field). Only one application was planned, but with a high infestation of corn earworms developing, two additional applications were made. The first was August 13, when pods averaged 1 inch in length, and the others August 28 and September 13. All materials reduced the lygus populations, but the differences between treatments and check were not significant (table 3). Poor control of both lygus and earworms may have been because of the low dosages. Only endrin and DDT were effective against the corn earworm. The other materials did not prevent excessive pod injury.

EXPERIMENTS IN 1955.—Seven materials were tested in a field of blackeye beans on the Griset farm, in plots 8 rows wide by 121 feet long, with six replicates. They were applied in sprays at 16 gallons per acre on August 1 and 15. Over a period of 29 days all materials gave significant reductions in populations of lygus (table 4). The beans in this field dried prematurely, so the lygus bugs left the field, and bean damage caused by pitting did not exceed 0.04%. All the insecticides except heptachlor, isodrin, and endrin gave small, insignificant increases in yield.

The same materials were tested on Ventura limas in plots 16 rows wide by 146 feet long, with eight replicates (Changula field). Sprays were applied August 4, when lygus populations ranged from 0.4 to 0.8 per sweep. All materials except isodrin gave significant reductions in lygus populations, but there were no significant differences between treatments (table 4). Owing to the premature drying of the plants less lygus damage than usual occurred—only 0.3% pitted beans in the untreated plots.

EXPERIMENTS IN 1956.—In the Griset field seven materials were tested as sprays on Ventura lima beans. Demeton was included because of its effectiveness in the control of the two-spotted spider mite. Plots were 8 rows wide by 100 feet long, with six replicates. The applications were made July 27 when lygus populations ranged from 0.4 to

Table 4.—Effect of various insecticides applied as sprays for the control of lygus bugs on lima beans, 1955.

| Insecticide and Pounds per Acre —Application | No. of Adults and Nymphs per Sweep | | Dry Beans per Acre (100-lb. sacks) |
|---|---|---|---|
| | Before Treatment[a] | After Treatment[b] | |
| *Griset Field (2 applications)* | | | |
| Dieldrin 0.8 | 4.1 | 0.17 | 21.2 |
| Endrin 0.8 | 2.6 | .25 | 18.2 |
| Aldrin 1.0 | 3.2 | .33 | 22.8 |
| Toxaphene 3.0 | 3.5 | .37 | 22.0 |
| DDT 1.5 | 3.5 | .48 | 20.0 |
| Isodrin 0.8 | 3.4 | .50 | 17.5 |
| Heptachlor 0.8 | 3.9 | .57 | 19.0 |
| Check | 4.4 | 1.20 | 19.6 |
| L.S.D. at 5% level | — | .34 | N.S. |
| *Changula Field (1 application)* | | | |
| Dieldrin 0.8 | 0.7 | 0.01 | |
| Endrin 0.8 | .8 | .04 | |
| Aldrin 1.0 | .5 | .02 | |
| Toxaphene 3.0 | .7 | .03 | |
| DDT 1.5 | .7 | .03 | |
| Isodrin 0.8 | .6 | .07 | |
| Heptachlor 0.8 | .7 | .03 | |
| Check | .4 | .11 | |
| L.S.D. at 5% level | — | .05 | |

[a] July 29 in Griset field, and Aug. 3 in Changula field.
[b] Aug. 1 to 30 in Griset field, and Aug. 5 to 25 in Changula field.

0.6 per sweep. For the 7-week period following the treatments all materials except demeton significantly reduced lygus populations below the check and all were about equal in effectiveness (table 5). In yield of beans and degree of injury no significant differences occurred between the treatments and check. Demeton was ineffective against lygus but was responsible for excellent yields because of its high acaricidal effect. However, all treated and untreated plots had high percentages of pitted beans.

Tests were continued on Ventura limas in the Casey field to evaluate the effectiveness of different insecticides against lygus when applied as sprays and dusts at the same dosages. Plots were 40 by 109 feet with six replicates. One application was made July 26 when lygus populations ranged from 0.4 to 1.3 per sweep. No significant differences were observed between dusts and sprays of dieldrin, DDT, and endrin in the reduction of lygus populations (table 5). The same result was true of yields, but all treated plots produced 1 to 4.6 hundred-pound sacks of beans per acre more than the check. Demeton did not give commercial control of lygus bugs, and of the materials used it had the highest percentage of pitted beans.

In a second experiment in the Casey field, one and two applications of the more promising materials were compared. Usually one application is not very effective when corn earworms are present and when lygus populations remain high until harvest time. Plots were 40 by 137 feet, with five replicates. Applications were made July 13, when blossoms and small pods were present, and August 16, when pods were 2 to 3½ inches long. After one treatment only dieldrin, demeton, toxaphene, and endrin gave significant reductions in lygus populations by August 15 (table 6). Endrin was the only material remaining effective until August 30, and demeton was the least effective.

**Table 5.—Effect of various insecticides applied as sprays and dusts for the control of lygus bugs on lima beans, 1956.**

| Insecticide and Pounds per Acre | No. of Adults and Nymphs per Sweep | | Beans Damaged by Lygus (%) | Dry Beans per Acre (100-Lb. Sacks) |
|---|---|---|---|---|
| | Before Treatment | After Treatment[a] | | |
| *Griset Field* | | | | |
| *Sprays* | | | | |
| Endrin 0.8 | 0.43 | 0.29 | 5.2 | 16.7 |
| Toxaphene 3.0 | .55 | .31 | 7.4 | 13.6 |
| Dieldrin 0.8 | .47 | .31 | 5.0 | 15.3 |
| DDT 1.5 | .61 | .32 | 6.8 | 16.8 |
| Heptachlor 0.8 | .47 | .32 | 7.2 | 15.6 |
| Aldrin 1.0 | .57 | .34 | 6.2 | 14.6 |
| Demeton 0.25 | .57 | .51 | 8.9 | 17.3 |
| Check | .52 | .61 | 9.7 | 15.2 |
| L.S.D. at 5% level | — | .19 | N.S. | N.S. |
| *Casey Field* | | | | |
| *Dusts* | | | | |
| Dieldrin 0.6 | 0.8 | .21 | 1.9 | 17.4 |
| DDT 2.2 | 0.9 | .38 | 3.4 | 16.7 |
| Endrin 0.6 | 0.8 | .43 | 3.1 | 15.8 |
| *Sprays* | | | | |
| Dieldrin 0.6 | 0.7 | .13 | 2.2 | 15.4 |
| DDT 1.7 | 1.0 | .27 | 1.7 | 16.8 |
| Endrin 0.6 | 0.4 | .37 | 2.8 | 18.7 |
| Demeton 0.25[b] | 1.3 | .59 | 6.8 | 16.0 |
| Check | 0.6 | .42 | 10.0 | 14.1 |
| L.S.D. at 5% level | — | N.S. | 2.6 | N.S. |

[a] July 28 to Sept. 14 in Griset field, and July 27 to Aug. 27 in Casey field.
[b] Demeton not included in analysis of dusts and sprays.

After two treatments, all but heptachlor gave significant reductions. With only one application there was no significant difference between any of the materials in the percentage of pitted beans, and the same was true of two applications of demeton and heptachlor. However, two applications of dieldrin, endrin, toxaphene, and DDT gave significant reductions below the checks.

Inspection of the threshed beans indicated that corn earworms were not a serious problem. To obtain some idea of the comparative damage shown by pods and threshed beans, 100 pods per plot were picked in the checks, and the beans in each inspected for injury. Of the 886 beans recovered in 500 pods, 13.1% were pitted, compared with 9.4% when threshed beans were examined. In addition, 3.4% of the beans were found to be injured by corn earworms, compared with 0.9% in the threshed samples. Apparently the threshed samples do not give a true picture of the damage done by lygus and corn earworms, because some of the damaged beans are removed in the threshing process.

Experiments in 1957.—Two experiments were conducted in the Casey field and one in the Beem field. In the first experiment in the Casey field, endrin, dieldrin, and the mixture of DDT-toxaphene were compared as sprays, in one, two, and three applications. Plots were 40 feet by 120 feet, with six replicates. Ventura lima beans were planted on May 24. Plots receiving only one application were sprayed July 23; those receiving two applications July 23 and August 7; and those receiving three July 5 (at time of blossoming), July 23, and August 7.

From the standpoint of lygus control, endrin was su-

**Table 6.—Effect of various insecticides applied as sprays for control of lygus bugs and corn earworms on lima beans, Casey field, 1956.**

| Insecticides and Pounds per Acre—Application | No. of Applications | No. of Lygus Adults and Nymphs per Sweep | | | Dry Beans per Acre (100-lb. Sacks) | Beans Pitted by Lygus (%) | Beans Damaged by Earworms (%) |
|---|---|---|---|---|---|---|---|
| | | Before Treatment | After First Treatment[a] | After Time of Second Treatment[b] | | | |
| Dieldrin 0.8 | 1 | 0.21 | 0.20 | 0.30 | 7.8 | 5.8 | 0.5 |
| | 2 | — | — | .08 | 10.1 | 4.5 | .7 |
| Demeton 0.25 | 1 | .31 | .26 | .72 | 10.4 | 7.7 | .7 |
| | 2 | — | — | .20 | 8.6 | 5.2 | .8 |
| Toxaphene 3.0 | 1 | .27 | .29 | .42 | 7.8 | 8.0 | .9 |
| | 2 | — | — | .08 | 5.5 | 4.3 | .7 |
| Endrin 0.8 | 1 | .17 | .29 | .24 | 10.9 | 6.0 | .7 |
| | 2 | — | — | .06 | 11.0 | 3.8 | .1 |
| Heptachlor 0.8 | 1 | .29 | .41 | .56 | 8.1 | 5.9 | .3 |
| | 2 | — | — | .32 | 10.5 | 6.0 | 1.3 |
| DDT 1.5 | 1 | .31 | .42 | .52 | 11.3 | 11.2 | .3 |
| | 2 | — | — | .08 | 11.6 | 3.7 | .1 |
| Check | | .30 | .54 | .76 | 13.3 | 8.5 | .4 |
| | | — | — | .70 | 9.2 | 9.4 | .9 |
| L.S.D. at 5% level | | — | .16 | .48 | n.s. | 4.3 | n.s. |

[a] Between July 13 and August 15.
[b] Between August 16 and August 30.

**Table 7.—Effect of various insecticides applied as sprays and dusts for the control of lygus bugs on lima beans, 1957.**

| Insecticide and Pounds per Acre—Application | No. of Applications | No. of Adults and Nymphs per Sweep | | Beans Damaged by Lygus (%) | Dry Beans per Acre (100-Lb. Sacks) |
|---|---|---|---|---|---|
| | | Before Treatment[a] | After Treatment[b] | | |
| *Casey Field, Experiment 1* | | | | | |
| *Sprays* | | | | | |
| Endrin 0.8 | 1 | 0.32 | 0.15 | 2.2 | 20.2 |
| | 2 | .24 | .23 | .8 | 19.2 |
| | 3 | .12 | .05 | .3 | 24.2 |
| Dieldrin 0.8 | 1 | .48 | .28 | 1.2 | 18.7 |
| | 2 | .36 | .18 | 2.8 | 16.5 |
| | 3 | .36 | .06 | .8 | 18.1 |
| DDT 1.5-toxaphene 3 | 1 | .40 | .21 | 1.0 | 20.7 |
| | 2 | .32 | .24 | .5 | 17.5 |
| | 3 | .20 | .09 | .9 | 20.3 |
| Check | | .24 | .37 | 6.3 | 17.3 |
| L.S.D. at 5% level | | — | .13 | 1.8 | n.s. |
| *Casey Field, Experiment 2* | | | | | |
| DDT | | | | | |
| In 10% dust 2.0 | | 0.57 | 0.40 | 3.8 | |
| In spray, | | | | | |
| 14 gal. 2.0 | | .60 | .49 | 3.5 | |
| 24 gal. 2.0 | | .50 | .51 | 4.6 | |
| 42 gal. 2.0 | | .43 | .38 | 3.6 | |
| 60 gal. 2.0 | | .43 | .62 | 4.3 | |
| Check | | .30 | .72 | 10.8 | |
| L.S.D. at 5% level | | — | n.s. | 2.6 | |
| *Beem Field* | | | | | |
| *Dusts* | | | | | |
| Endrin 0.84 | | 0.4 | 0.21 | 0.2 | |
| Dieldrin 0.75 | | .1 | .22 | .3 | |
| Toxaphene 4.0 | | .3 | .26 | .5 | |
| DDT 1.5-toxaphene 4.5 | | .2 | .29 | .4 | |
| DDT 1.6 | | .3 | 1.43 | 1.6 | |
| Delnav 0.72 | | .4 | 2.0 | — | |
| Trithion 0.96 | | .4 | 2.3 | — | |
| Check | | .3 | 3.1 | 4.2 | |
| L.S.D. at 5% level | | — | .8 | n.s. | |

[a] June 27, Casey Expt. 1; July 26, Casey Expt. 2; Aug. 2, Beem.
[b] Between July 6 and Aug. 21, Casey Expt. 1; between Aug. 6 and Sept. 18, Casey Expt. 2; between Aug. 6 and Sept. 3, Beem.

perior, because all applications had significantly lower populations than the check (table 7). One application of dieldrin and two of DDT-toxaphene were not significantly better than the check. In yield of lima beans, all treatments except dieldrin (two applications) exceeded the check, but the differences were not significant. Endrin and DDT-toxaphene were somewhat more effective than the other materials, producing higher yields. On the basis of injury to beans taken from pods, no significant differences occurred between materials or number of applications, but all materials reduced the number of pitted beans below the check.

In the second experiment in the Casey field, comparison was made of DDT at 2 pounds per acre used as a dust and also in sprays at different gallonages per acre. One application was made August 1, when pods were 1 to 2 inches long. Plots were 40 by 139 feet, with six replicates.

Weekly lygus surveys showed that neither sprays nor dusts were highly effective (table 7). With most of the treatments there was a gradual increase in population on August 14 and an additional increase on August 18, only 17 days from the time of application. On this date, however, all treatments had significantly lower populations than the check.

Analysis of the adult lygus populations for the period July 16 to September 18 showed significant reductions with the 10% dust and the 42-gallon spray. In the control of adults and nymphs and of nymphs alone no significant differences were observed between treatments and check. The 60-gallon spray was too dilute, being inferior to the dust and also to the lower-gallonage sprays. In bean injury caused by lygus, all treated plots had a significantly lower percentage of pitted beans, but the differences between treatments were not significant.

Dusts were tested on Fordhook beans in the Beem field in plots 35 by 103 feet, with six replicates. Two acaricides, Delnav® and Trithion®, were included in these tests. The beans were planted June 18 and the dusts applied August 5 when pods were 1 to 2 inches long. With the exception of the acaricides and 10% DDT, good control was obtained with all materials for 3 weeks (table 7). For some reason DDT by itself failed to give adequate control, and because of the excessive lygus buildup these plots and those treated with acaricides were re-treated with 10% DDT August 29. In bean damage no significant difference occurred between the treatments and the check, but all materials reduced the percentage of pitted beans.

SYSTEMIC INSECTICIDES IN THE SOIL AGAINST LYGUS BUGS, 1957.—Systemic insecticides applied to the soil as side dressings were tested in two fields (Beem and Casey) in plots 4 rows wide by 87 feet long with five replicates. The emulsions were applied 5 inches deep and 5 inches to one side of the bean row with a tractor equipped with four injection blades and a pump attached to the power takeoff, at the rate of 10 gallons per acre. In the Beem field Fordhook beans were planted June 15, the treatments made July 16, and the beans were irrigated 7 days after treatment. In the Casey field Ventura beans were planted May 25, and the treatments made July 18, but the beans were not irrigated. Lygus populations were determined weekly until harvest (table 8). These systemics at the dosages used gave no control of adults or nymphs either initially or throughout the season. The Beem field became so heavily infested with lygus bugs that it was necessary to dust the entire layout with DDT on August 29.

EXPERIMENTS IN 1958.—In the Casey field, Sevin®, ethion, and Thiodan® were compared with the older materials against lygus bugs and corn earworms. Plots were 40 by 109 feet, with six replications. Applications were made July 11, when blossoms and small pods were present, and to some of them again July 24. All materials were applied in sprays at 22 gallons per acre.

Against adults dieldrin was the most effective (table 9). The one application of DDT-toxaphene was the least effective, but two applications were very good. All other materials at one or two applications gave significantly lower populations than the check.

Dieldrin was also the most effective against nymphs, and ethion and Sevin were least effective. All treatments except one application of DDT-toxaphene had significantly lower nymph populations than the check. In the control of both adults and nymphs, dieldrin, endrin, Thiodan, and two applications of DDT-toxaphene were the most effective. Dieldrin greatly reduced pitting, but was the least effective in preventing damage caused by corn earworms. Sevin gave the highest percentage of pitted beans. All other materials reduced the pitting, and two applications were generally superior to one in this respect. Pod inspection to determine damage caused by corn ear-

**Table 8.—Effect of systemic insecticides applied in the soil on lygus populations, Beem and Casey fields, 1957.**

| Insecticide and Pounds per Acre | No. of Lygus Bugs per Sweep (July 22–Aug. 26) | |
|---|---|---|
| | Adults | Nymphs |
| *Beem Field, Fordhook Beans* | | |
| Di-Syston® | | |
| 0.5 | 0.8 | 1.0 |
| 1.0 | 1.0 | 1.0 |
| 2.0 | .9 | 1.2 |
| Phorate | | |
| 0.5 | .8 | 0.9 |
| 1.0 | .9 | 1.1 |
| 2.0 | .8 | 1.0 |
| Check | .8 | 1.0 |
| L.S.D. at 5% level | N.S. | N.S. |
| *Casey Field, Ventura Beans* | | |
| Phorate | | |
| 1.0 | 0.7 | 0.25 |
| 2.0 | .8 | .24 |
| Guthion® | | |
| 1.0 | .6 | .24 |
| 2.0 | .5 | .22 |
| Demeton | | |
| 1.0 | .7 | .30 |
| 2.0 | .7 | .23 |
| Phosdrin® | | |
| 1.0 | .8 | .33 |
| 2.0 | .6 | .23 |
| Check | .8 | .31 |
| L.S.D. at 5% level | n.s. | n.s. |

worms showed Thiodan and DDT the most effective, and dieldrin least effective.

Another experiment was conducted in the Beem field to further evaluate Sevin and ethion against lygus. Plots were 40 by 110 feet with six replicates. One and two applications were made at the rate of 13 gallons per acre, the first July 8 when blossoms and small pods were present, and the second July 22, when pods were 1 to 2½ inches long. In one plot a single application was made July 22.

**Table 9.—Effect of various insecticides applied in sprays for the control of lygus bugs and corn earworms on lima beans, Casey field. 1958.**

| Insecticide and Pounds per Acre —Application | No. of Applications | No. of Lygus Bugs per Sweep After 7/25 and 9/4 | | Beans Damaged by Lygus (%) | Pods Damaged by Earworms (%) |
|---|---|---|---|---|---|
| | | Adults | Nymphs | | |
| Dieldrin 0.8 | 1 | 0.06 | 0.0 | 0.3 | 5.4 |
| | 2 | .03 | .0 | .6 | 3.2 |
| Endrin 1.0 | 1 | .13 | .04 | 2.5 | 2.6 |
| | 2 | .08 | .07 | .9 | 0.0 |
| Thiodan 1.6 | 1 | .15 | .06 | .8 | .4 |
| | 2 | .10 | .07 | .5 | .4 |
| DDT 1.4-toxaphene 1.8 | 1 | .24 | .19 | 1.3 | 2.0 |
| | 2 | .09 | .03 | .9 | 2.2 |
| DDT 1.5 | 1 | .10 | .17 | 2.3 | 1.2 |
| | 2 | .14 | .16 | 2.4 | .4 |
| Ethion 2.0 | 1 | .13 | .19 | 3.3 | 1.0 |
| | 2 | .17 | .17 | .6 | 2.4 |
| Sevin 1.8 | 1 | .13 | .25 | 3.9 | .8 |
| | 2 | .16 | .20 | 5.0 | 1.8 |
| Check | — | .29 | .56 | 6.4 | 1.0 |
| L.S.D. at 5% level | — | .12 | .22 | 3.3 | 2.8 |

**Table 10.—Effect of insecticides applied as sprays against lygus bugs on lima beans. Callens farm, 1959.**

| Insecticide and Pounds per Acre —Application | No. of Applications | No. of Lygus Bugs per Sweep July 16–Aug. 12 | | Beans Damaged by Lygus (%) | Dry Beans per Acre (100-Lb. Sacks) |
|---|---|---|---|---|---|
| | | Adults | Nymphs | | |
| DDT 2 | 1 | 0.05 | 0.05 | 1.5 | 11.7 |
| | 2 | .05 | .0 | 2.0 | 14.2 |
| DDT 4 | 1 | .03 | .04 | .8 | 13.4 |
| | 2 | .03 | .0 | 1.5 | 13.3 |
| Toxaphene 2 | 1 | .07 | .07 | 1.2 | 13.1 |
| | 2 | .05 | .03 | 2.2 | 12.5 |
| Toxaphene 4 | 1 | .03 | .0 | 2.5 | 12.8 |
| | 2 | .03 | .01 | 1.0 | 13.7 |
| DDT 1-Toxaphene 1 | 1 | .07 | .05 | 2.2 | 10.7 |
| | 2 | .07 | .0 | 1.3 | 13.9 |
| DDT 2-Toxaphene 2 | 1 | .05 | .04 | 1.8 | 13.3 |
| | 2 | .05 | .01 | .8 | 13.6 |
| Sevin 2 | 1 | .10 | .03 | 1.5 | 13.7 |
| | 2 | .09 | .02 | 1.2 | 13.0 |
| Check | | .22 | .19 | 5.3 | 13.8 |
| L.S.D. at the 5% leve | | .07 | .05 | 1.3 | n.s. |

Lygus were not a serious problem in this field. In populations of adults and nymphs and of adults alone, no significant differences were apparent, but in nymph control one and two applications of DDT-toxaphene, endrin, and one application of ethion gave significant reductions below the check. Two applications were slightly better than one. Sevin was the least effective. In bean injury no significant difference occurred between the treatments and check.

EXPERIMENTS IN 1959.—In the Callens field DDT and toxaphene alone and in combination were compared as sprays in one and two applications at two dosages. Sevin was also included. Plots were 40 by 143 feet, with six replicates. Ventura lima beans were planted April 29. Plots receiving one application were treated July 8, and those receiving two applications July 8 and 27.

In the control of adults and nymphs all treatments gave significant reductions below the check (table 10). No significant difference was evident between materials, dosages, or number of applications. Two applications were generally somewhat more effective than one. Sevin was more effective against nymphs than against adults. All materials reduced the percentage of pitted beans below the check. With DDT inconsistencies appeared, more pitting occurring with two applications than with one. No marked difference occurred in the degree of pitting between DDT and toxaphene at the dosages and number of applications used. In yield of lima beans there was no significant difference between materials and the check.

DISCUSSION.—A compilation of the average lygus populations, yield, and injury data for the more promising materials tested shows that from the standpoint of lygus control, dieldrin was superior, followed by toxaphene, endrin, and DDT-toxaphene (table 11). DDT used by itself ranked sixth. In reducing bean pitting caused by lygus, DDT-toxaphene was the most effective, but the results were based on fewer tests. DDT-toxaphene also gave the

Table 11.—Average lygus populations in southern California, bean injury, yield, and cost of insecticides used in experiments, 1953–1958.

| Insecticide and Pounds per Acre | Lygus Bugs per Sweep after Treatment | | Beans Pitted (%) | Dry Beans per Acre (100-Lb. Sacks) | Cost of Material per Acre[a] |
|---|---|---|---|---|---|
| | Adults | Nymphs | | | |
| Dieldrin 0.8 | 0.16 | 0.01 | 1.6 | 17.0 | $3.78 |
| Toxaphene 3.9 | .27 | .03 | 2.8 | 14.4 | 3.02 |
| Endrin 0.9 | .28 | .03 | 2.0 | 16.9 | 7.31 |
| DDt 1.5-toxaphene 3 | .24 | .19 | 0.7 | 18.3 | 3.88 |
| Heptachlor 1.1 | .23 | .04 | 4.3 | 16.1 | 3.88 |
| DDT 2.0 | .24 | .06 | 2.3 | 16.4 | 2.06 |
| Check | .36 | .16 | 4.9 | 14.8 | — |

[a] 1958 prices on the basis of 30-gallon drums of emulsion concentrate.

highest yield of beans (18.3 sacks per acre). For all materials the average yield of lima beans was 16.5 sacks per acre compared with 14.8 for the checks. This increase of 1.7 sacks of beans per acre, of better quality, with a monetary value of $20, would more than compensate for the added expense of making one or even two applications of any of the more promising insecticides used in these tests.

In judging the relative merits of these insecticides, one must take into account their effectiveness against both lygus and corn earworms. Dieldrin and heptachlor did not give satisfactory control of earworms. Endrin gave exceptionally good control of both insects, but it is rather costly. Less expensive materials are toxaphene, DDT-toxaphene, or DDT alone, with the last mentioned the most economical to apply. Unless resistance by lygus develops, 2 pounds of DDT per acre in either a spray or dust will give satisfactory control of lygus if applied near the termination of blossoming. A second application 2 to 3 weeks later will reduce damage to pods and beans caused by corn earworms, and will prevent reinfestation from lygus nymphs hatching from eggs deposited by adults before treatment. Toxaphene and DDT-toxaphene have shown considerable promise, but more extensive tests will be necessary to evaluate fully their effectiveness against both lygus bugs and corn earworms.

### Chemical Names of the Proprietary Materials Tested

Aramite 2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite

Chlorobenzilate-ethyl 4,4′-dichlorobenzilate

Delnav 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)

Diazinon *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phorphorothioate

Di-Syston *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate

Guthion *O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate

Phosdrin 1-methyoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical

Sevin *N*-methyl 1 naphthyl carbamate

Thiodan 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide

Trithion *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate

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# Brief History of Aphid Control on Potatoes in South Florida, 1946–59[1]

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### Abstract

Results are given from 13 seasons of studies for control of the green peach aphid, *Myzus persicae* (Sulz.), on potatoes. Control of the aphid was often achieved initially and effectively with an insecticide, only to be reduced within a few years so that it was superseded by another toxicant. DDT emulsion sprays gave satisfactory control for about 5 years. Parathion gave satisfactory aphid control for about 10 years and was effective for control of other insects infesting potato plants. Toxaphene was effective in many seasons. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) is currently recommended for control of the aphid.

Over the last 13 seasons, 1946–59, the green peach aphid, *Myzus persicae* (Sulz.), has been one of the two most injurious insect pests infesting potato plants in south Florida. Comparative injuriousness of the green peach aphid and of the serpentine leaf miner, *Liriomyza pusilla* (Meig.), was shown by a regression study in which yields of potatoes were affected by aphids more than by leaf miners (Wolfenbarger 1954). A brief summary of field tests by Wolfenbarger (1958) showed that in initial tests new insecticides were often effective in controlling the serpentine leaf miner but frequently such materials become ineffective within a very few years. Similar results were obtained with the green peach aphid and the results are summarized below.

Potatoes are usually planted between late October and mid-December in south Florida and are harvested approximately 3 months later. Aphid infestations appeared about the last week in January from 1946 through the 1957 season. Infestations appeared during the last week in December for the 1957–58 season and the last week in November for the 1958–59 season. Control of the aphid by growers was more difficult during the last 2 than during the previous 11 years.

MATERIALS AND METHODS.—Field plot experiments were used in which plants were sprayed by power equip-

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**Table 1.—Evaluation of insecticides in control of the green peach aphid, at Homestead, Florida. 1958–59.**

| Insecticides | Amount in 100 Gal./A. | No. Aphids/ Leaf[a] Feb. 3 | |
|---|---|---|---|
| Thiodan 25%, W.P. | 2 lb. | 2 | ] |
| Phorate, 2 E.C. | 1 pt. | 2 | ] |
| Phosphamidon, 4 E.C. | 1 pt. | 5 | ] |
| Bayer 25141 50%, W.P. | 0.5 lb. | 6 | ] |
| Toxaphene, 6 E.C. | 0.16 gal. | 20 | ] |
| Phosdrin 25%, W.P. | 1 lb. | 26 | ] ] |
| Toxaphene 40%, W.P. | 2 lb. | 37 | ] ] |
| Toxaphene, 8 E.C. | 1 pt. | 40 | ] ] |
| Toxaphene 40%, W.P. | 2 lb. | 43 | ] ] |
| Diazinon, 2 E.C. | 1 pt. | 52 | ] ] |
| Parathion 15%, W.P. | 2 lb. | 105 | ] ] |
| Toxaphene 40%, W.P. | 2.5 lb. | 144 | ] ] |
| Check | | 145 | ] ] |
| Bayer 29493 50%, W.P. | 0.5 lb. | 166 | ] |
| Dibrom, 8 E.C. | 0.5 pt. | 167 | ] |
| Kepone 50%, W.P. | 2 lb. | 324 | ] |

[a] Means paralleled by the same lines are not significantly different at the 5% level.

ment with booms having nozzles directed to the sides and tops of plants. Insecticides were combined in the spray tank with the recommended fungicides. Check treatments received applications of fungicides but without any insecticide. In order to obtain thorough spray coverage to protect the plants from late blight infestations high gallonages of 75 to 140 per acre were applied. There were four replications of each treatment arranged in randomized blocks, except in cooperative tests with growers. Each replication was four or more rows wide and 36 or more feet in length.

Amounts of insecticides given below refer to the quantity of the formulation in 100 gallons of the spray mixture. Insecticides were applied to the experimental plants at weekly intervals during periods when insects were present, except in cooperative tests with growers in 1957–58. In these cooperative tests only one application was made.

Data were usually taken on two or more occasions for each experiment. The aphid count was usually made on the fourth or fifth leaf from the bottom of the plants and about 3 to 6 days following the previous spray application. Four leaves per treated plot was the usual number sampled. Although aphids per leaf were counted, the results are expressed as percentages of control Abbott (1925).

Insecticides were frequently tested one or two seasons then dropped from further experimentation. Sometimes a test material was effective for control of one insect but not for another. Sometimes an insecticide was sufficiently effective to be tested a second or third season but was dropped in the insuing tests.

Results.—The new materials tested each year are indicated with a brief statement concerning the results. A tabular arrangement, table 1, is given to show the 1958–59 results. Chemical definitions of materials not bearing adopted common names appear at the end of this paper.

The first data were taken during the 1945–56 seasons. These data showed that 50% DDT, 2 lb. WP (wettable powder), 2 lb. or 2 EC (emulsion concentrate), 1 qt., was effective in controlling the green peach aphid. Nicotine sulfate, 40%, 1 pt., per 100 gal. spray, gave 39% control, BHC, 2 lb. of 7.5% gamma isomer WP gave 65% control and Lethane B-72® 13.5%, 3 lb. WP, gave 25% control, after one application.

In 1946–47 chlordane and TEPP were the new materials tested. Chlordane, 8 EC, 1 qt., TEPP, 50% active ingredient (according to the supplier), 1 qt., each gave 66% control. BHC, 10% gamma isomer WP, 2 lb. gave almost 100% control, whereas nicotine sulfate, 40% 1 pt. combined with 3 lb. lead arsenate gave 88% control. DDT, 50%, 4 lb. WP, gave perfect control where 72 aphids per leaf was the average on the check plots.

In 1947–48 toxaphene and parathion were the new insecticides tested. Emulsions and wettable powder formulations of DDT, chlordane, lindane, and toxaphene were compared. Emulsion formulations were more effective than wettable powders, except with chlordane in which the emulsion was the less effective. Parathion 25%, 2 lb., WP, was the most effective aphicide tested although HETP and TEPP were very effective.

In 1948–49 aldrin was first tested but gave poor control. Calcium arsenate 3 lb. combined with TEPP, 40 EC, one-half pt., was included in the tests, and gave 87% control.

In 1949–50, dieldrin 15%, 1 lb. WP, and Dilan 25 EC, 1 qt., were tested against the aphid on potatoes. Both phosphatic materials and Dilan were very effective in reducing the aphids. A 61% control was obtained from dieldrin. Toxaphene EC gave 97% control, and the WP formulation gave 86% control. Toxaphene was believed, however, to have injured plants and to have reduced the yields slightly.

In 1950–51 malathion and demeton (Systox) in initial tests were highly effective. Toxaphene, EC and WP formulations, was not effective in the early part of the season and plants sprayed with it had more aphids in the latter part of the season than the check. Yields were believed slightly reduced by these formulations. DDT, 2 EC, 1 qt., was effective in the early part of the season but plants sprayed with it had more aphids than the check in the latter part of the season.

In the 1951–52 season wettable powder formulations of endrin and isodrin were first tested. Endrin suppressed the aphid population; isodrin was comparatively ineffective. The phosphatic materials, malathion, EPN, TEPP and Metacide® were very effective.

Foliar spray experiments were not conducted in the 1953–56 seasons. During this time parathion was used successfully by growers. DDT emulsion was used occasionally for control of lepidopterous larvae but was not satisfactory for control of the green peach aphid. Toxaphene was used occasionally but plants sprayed with it frequently became yellowed. Parathion controlled the serpentine leaf miner and suppressed other insect pests of the potato plants.

In 1956–57 ethion, Guthion®, phorate, and Thiodan® were initially tested as foliar sprays. These gave control as follows: ethion—79%, Guthion—52%, phorate—99% and Thiodan—nearly 100%. Two pounds of 15% parathion WP gave 98% control. There were reports from growers, however, that parathion, TEPP, DDT, and toxaphene were not effective. (Phorate has approval for preplanting as a soil treatment only.)

In the 1957–58 season the green peach aphid appeared the last week in December, a month earlier than previ-

Table 2.—Evaluations of DDT, parathion, and toxaphene over the years tested at Homestead, Florida, against the green peach aphid on potatoes.

| YEAR | DDT | PARATHION | TOXAPHENE |
|---|---|---|---|
| 1945–46 | 99 | | |
| 1946–47 | 99 | | |
| 1947–48 | 37 | 88 | 66 |
| 1948–49 | 98+ | 99+ | 88 |
| 1949–50 | | 99+ | 92 |
| 1956–57 | More than check | 98[a] | |
| 1957–58 | More than check | 60[b] | |
| 1958–59 | More than check | 28[a] | 56 |

[a] Two pounds of WP, 15%, used per 100 gallons water.
[b] One pint of 4 EC used per 100 gallons water.

ously observed. In late January growers began reporting lack of control by sprays that previously had given satisfactory control. New materials tested, all in cooperative tests with growers, were: 25% Diazinon® WP, 1½ lb.; Phosdrin®, 2 EC, 1 pt.; phosphamidon, 4 EC, ½ pt.; methyl parathion 2 EC, 1 pt.; Di-Syston®, 2 EC, 1 pt.; Sevin® 25%, 1 lb. WP, and Trithion®, 4 EC, 1 pt. Of these and other materials tested, Thiodan 25%, 2 lb. WP, and 2 EC, 1 qt., was the most effective aphicide while Phosdrin ranked a rather poor second in effectiveness. These data were taken from 2 to 3 days after single applications of the insecticide.

New materials tested in 1958–59 were Bayer 25141 and Bayer 29493, each 50% WP and tested at ½ lb., Dibrom®, 8 EC, ½ pt., dimethoate, 2 EC, 1 pt., 50% Kepone® WP, 2 lb. and phosphamidon 4 EC, 1 pt., and silica jel materials. Thiodan was widely used by growers with satisfactory results. Toxaphene emulsions tended to give a little more effective aphid control and a little higher yield than wettable powder formulations. Silica jels, two formulations, applied at 20 pounds per acre by a powder duster, appeared to make no reduction in the aphid populations.

Several new materials were included in the experiments in 1958–59 (table 1). Considerations in the test use of these included the control of insects other than the green peach aphid.

Thiodan was the most effective aphicide and is currently recommended. Although phorate was tested as a foliage spray material and was very effective it has label approval only as a preplanting soil treatment. Other treatments are listed in order of average number of aphids per leaf and show that Kepone had significantly more aphids than the check, owing perhaps to reducing the number of predators and parasities without reducing the aphid population.

DISCUSSION OF RESULTS.—Tests made with pre- and post-DDT insecticides for green peach aphid control during 1946–59 showed that many chemicals gave some measure of control. Initial tests showed that DDT and other insecticides were often effective whereas they frequently were unsatisfactory in later seasons. This is shown by changes in the degree of control obtained over the years as shown in table 2. Although this ineffectiveness very likely indicates tolerance or resistance to the toxicant by the insect, there is lack of evidence to make such a statement. The chlorinated hydrocarbon toxicants were generally less effective than the organophosphorous chemicals. Toxaphene was effective as an aphicide but occasionally produced yellowing of plants and reduced yields. Recent tests with toxaphene suggest that improvements in formulation have been made so that yields are not reduced by its use. Further experimentation with toxaphene seems to be justified. Parathion was the most effective aphicide for a decade, suppressed other insects infesting potatoes, and was therefore used widely and intensively. Thiodan was found effective in aphid control about the time parathion became ineffective and is currently recommended for use.

Chemical definitions of materials not bearing adopted common names:

Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)
Diazinon (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate)
Dibrom (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate)
Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane)
Di-Syston (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)
Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate)
Kepone (1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$ .$0^{3,9}$ .$0^{5,8}$] decan-4-one)
Lethane B-72 (n-butylcarbitol thiocyanate)
Metacide (A formulation of methyl parathion and parathion)
Phosdrin (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical)
Sevin (1-naphthyl-*N*-methyl carbamate)
Thiodan (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9,methano-2,4,3-benzodioxathiepin-3-oxide)
Trithion (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)

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# Systemic Insecticides for Control of Scales, Leaf Miners, and Lace Bugs[1]

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ABSTRACT

Soil drenches of phorate and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) were more effective than Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethylphosphorodithioate), and Sytam® (4 pounds per gallon of schradan) in controlling scale on holly (*Asterolecanium* sp.). Pine needle scale (*Phenacaspis pinifoliae* (Fitch)) was controlled with phorate granules. Dimethoate granules were more efficient than phorate in killing first-brood birch leaf miners (*Fenusa pusilla* (Lep.)), but less so on the second brood. Dimethoate granules controlled leaf miners in holly (*Phytomyza ilicis* (Curt.)) and boxwood (*Monarthropalpus buxi* (Lab.)) and lace bugs (*Stephanitis* spp.) on andromeda, azalea, and rhododendron. Phosphamidon gave poor control of leaf miners but good control of lace bugs.

The possibility of controlling some leaf miners on ornamental plants by applications of systemic insecticides has been demonstrated in preliminary experiments by (Schread 1958) and confirmed (Schread 1959). Beckwith & Duda (1959) have reported on their experiments in controlling holly leaf miner with soil treatments of 2% phorate granules. This type of treatment has the obvious advantage of simplicity of application, easy timing, and effectiveness over long periods. Phorate was used in the earlier tests, but its use for this purpose has not yet been approved.

The present series of tests was made to extend our knowledge of the effectiveness of several additional systemic insecticides on the following pests:

*Asterolecanium* sp., scale on holly
*Phenacaspis pinifoliae* (Fitch), pine needle scale
*Fenusa pusilla* (Lep.), birch leaf miner
*Phytomyza ilicis* (Curt.), holly leaf miner
*Monarthropalpus buxi* (Lab.), boxwood leaf miner
*Stephanitis globulifera* (Mat.), andromeda lace bug
*Stephanitis pyrioides* Scott, azalea lace bug
*Stephanitis rhododendri* Horv., rhododendron lace bug

MATERIALS AND METHODS.—The insecticides used were: dimethoate 5%, phosphamidon 2%, phorate 2% granules and phosphamidon 46% and phorate 47% emulsions, in addition to Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), Sytam® (4 pounds per gallon of schradan), and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate).

CONTROL OF HOLLY SCALE.—On July 19, 1957, 18″ scale-infested holly plants in 4″ pots were given soil drenches of phorate, Phosdrin, Di-Syston, and Sytam at dilutions of 1 to 400 and 1 to 600 of formulation. Each pot received about 8 ounces of drench. Each treatment consisted of four plants and six untreated checks. Counts of young scales before treatment indicated an average of 6.5 per leaf in a five-leaf sample per plant.

Data on control were taken on August 6 by counting dead and live scales on five leaves taken at random from each treated plant and from the untreated checks. Results given in table 1 show that phorate and Phosdrin controlled the scales but Di-Syston and Sytam were inefficient.

CONTROL OF PINE NEEDLE SCALE.—In a preliminary experiment on August 22, 1957, phorate granules were applied and raked into the soil under 18″ Mugho pines at the rate of 1¾, 3½, and 5½ ounces of formulation per 10 sq. ft. under each plant to obtain the amounts of technical phorate shown in table 2. Data on control were recorded

**Table 1.—Control of holly scale in pots treated with soil drenches, August 6, 1959.**

| MATERIAL AND DILUTION | SCALES | | PER CENT CONTROL |
|---|---|---|---|
| | Dead | Alive | |
| Phorate 1–800 | 96 | 0 | 100 |
| Phosdrin 1–800 | 119 | 0 | 100 |
| Di-Syston 1–400 | 34 | 32 | 51.5 |
| Di-Syston 1–800 | 62 | 42 | 59.6 |
| Sytam 1–400 | 36 | 96 | 27.2 |
| Sytam 1–800 | 44 | 84 | 34.3 |
| Untreated | | 133 | |

**Table 2.—Control of pine needle scale with granules applied to the soil, September 8, 1959.**

| TECHNICAL PHORATE PER PLANT (OZ.) | SCALES | | PER CENT CONTROL |
|---|---|---|---|
| | Dead | Alive | |
| 0.035 | 38 | 281 | 11.9 |
| .070 | 13 | 61 | 17.5 |
| .105 | 40 | 49 | 44.9 |
| Untreated | | 152 | |

on September 8 by counting dead and live scales on 50 pine needles taken at random from five 3″ twigs per rate of treatment and from the check. As shown by the table, phorate used at the rates indicated did not give good control of the pine needle scale.

CONTROL OF BIRCH LEAF MINER.—On April 24, 1959, 7′- to 9′-clump white birch trees, with two to four stems per clump, growing at 3′ intervals in 6′ nursery rows were treated with granules of phorate, phosphamidon, and dimethoate applied by hand as a side dressing. There were 27 clumps of birch per row and only 15 rows available for the tests. Rates of 12, 24, and 36 pounds of technical phorate were applied per acre by applying 20, 40, and 60 pounds of a 2% formulation, each to a three-row plot. Rates of 12, 24, and 48 pounds of technical dimethoate and phosphamidon were achieved per acre by applying 2.7, 5.4, and 10.8 pounds of a 5% formulation of the former and 6.66, 13.32, and 26.64 pounds of a 2% formulation of the latter systemics, each to one row. The soil was cultivated at 5- to 7-day intervals from the end of April through early August. Results of the treatments are given in table 3. Twenty-five leaves were taken at random

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**Table 3.—Control of birch leaf miner granules applied to the soil. 1959.**

| Toxicant per Acre (lb.) | First Brood June 3 | | Second Brood July 6 | |
|---|---|---|---|---|
| | Total Larvae | Per Cent Kill | Total Larvae | Per Cent Control |
| Phorate 12 | 191 | 59.1 | 138 | 100.0 |
| 24 | 247 | 87.0 | 138 | 100.0 |
| 36 | 205 | 90.7 | 146 | 93.8 |
| Dimethoate 12 | 340 | 97.6 | 140 | 71.4 |
| 24 | 353 | 100.0 | 213 | 75. |
| 48 | 375 | 100.0 | 229 | 91.7 |
| Phosphamidon 12 | 239 | 5.0 | 122 | 27.8 |
| 24 | 281 | 14.9 | 97 | 23.7 |
| 48 | 184 | 68.4 | 257 | 19.6 |
| Untreated | 265 | | 137 | |

per treatment and the dead and live miners counted. As indicated, phorate and dimethoate gave good to excellent control of both broods of birch leaf miner, whereas phosphamidon gave mostly poor results. Perhaps an explanation of the lag in control of the first brood of miners by phorate may lie in the dryness of the soil in the phorate-treated rows and the quantity of rain required to obtain a comparable absorption of the systemic by the trees. Control of the second brood was considerably better with phorate than with dimethoate. Dimethoate caused injury to the leaves, most noticeable at 48 pounds per acre, less at 24, and not evident at 12.

CONTROL OF HOLLY LEAF MINER.—*Ilex opaca*, averaging 4′ high, were given soil treatments of $\frac{1}{4}$ and $\frac{1}{2}$ pound of dimethoate granules and $\frac{1}{2}$ and 1 pound of phosphamidon on July 23. The granules were raked into the soil in an area of 9.4 sq. ft. per plant. No watering was done. Each treatment consisted of three treated and six untreated plants. Control data were taken on September 2 by dissecting the mines in 20 leaves taken at random from the treated and untreated plants (table 4). Dimethoate was much more effective than phosphamidon.

CONTROL OF BOXWOOD LEAF MINER.—On July 15, 1959, dimethoate granules were applied to 6.2 sq. ft. of soil under $2\frac{1}{2}$′ boxwoods (*Buxus sempervirens*) and phosphamidon granules were applied on July 22 at the rate of $\frac{1}{4}$, $\frac{1}{2}$, and 1 pound of formulation per plant. The soil was watered several times for a week. Control data were taken August 24 by dissecting 20 leaves taken at random from

**Table 4.—Control of holly leaf miner with granules applied to the soil, September 2, 1959.**

| Toxicant per Plant (oz.) | Miners | | Per Cent Control |
|---|---|---|---|
| | Dead | Alive | |
| Dimethoate 0.012 | 123 | 0 | 100.0 |
| .025 | 166 | 0 | 100.0 |
| Phosphamidon .01 | 33 | 78 | 29.7 |
| .02 | 40 | 123 | 24.5 |
| Untreated | | 144 | |

**Table 5.—Control of boxwood leaf miner with granules applied to the soil. August 24, 1959.**

| Toxicant per Plant (oz.) | Miners | | Per Cent Control |
|---|---|---|---|
| | Dead | Alive | |
| Dimethoate 0.05 | 49 | 0 | 100.0 |
| .025 | 32 | 42 | 43.2 |
| .012 | 4 | 101 | 3.8 |
| Phosphamidon .02 | 39 | 36 | 52.0 |
| .01 | 0 | 142 | 0.0 |
| .005 | 0 | 138 | 0.0 |
| Untreated | | 87 | |

each treatment. The results (table 5) indicate that dimethoate was most effective at the highest dosage, whereas phosphamidon gave poor to no control of the boxwood leaf miner.

CONTROL OF ANDROMEDA LACE BUG.—On July 14, 1959, 32″ andromedas (*Pieris japonica* (Thumb)) were given a 1-gallon soil drench of phosphamidon emulsion at dilutions of 1 to 200 and 1 to 400 of formulation applied to 7.8 sq. ft. under each plant. There were three plants and untreated checks in each test. On July 27, phosphamidon granules were raked 2″ into the soil under 42″ andromedas at the rate of $\frac{1}{2}$ and 1 pound of formulation to 14 sq. ft. under each plant. Each treatment was repeated twice. Pretreatment counts indicated an average of 202 adults on 140 leaves per plant. Dimethoate granules were used in an additional test on July 16. One-quarter and $\frac{1}{2}$ pound of formulation was raked 2″ into 7.8 sq. ft. of soil under 32″ andromedas. The treatments were repeated three times. All treated areas were soaked with water when granules were applied and twice in addition at 2-day intervals. All the above tests were evaluated on August 4 by 5-minutes-per-plant examination of the andromedas for live adult lace bugs. In addition a more thorough search was made for live nymphs by examining about 150 leaves per treatment. The results showed that no live lace bugs occurred on any of the treated plants, whereas 311 live adults were found on the untreated ones. Numerous live nymphs on the untreated plants were not counted.

CONTROL OF AZALEA LACE BUG.—Plants of *Azalea obtusum amaenum* and *A. hinodegiri* averaging 4′ high were treated during the second half of July 1959 with dimethoate granules at $\frac{3}{4}$ and $1\frac{1}{2}$ pounds of formulation and with phosphamidon granules at $1\frac{1}{2}$ and 3 pounds of formulation to 20.8 sq. ft. under each plant to obtain the levels of technical systemics shown in table 6. There were three plants per treatment divided equally between the two varieties of azaleas, and four untreated ones. The azaleas were growing in sod, hence the granules were not raked into the ground. The soil was soaked with water when the granules were applied and three times during the following week. Adult lace bugs only were counted for control data. This count was made by shaking the adults from 10 separate areas of a plant onto a 20″×30″ piece of white cloth. These data appear in table 6. It is clear that comparable amounts of technical dimethoate gave complete control of azalea lace bugs sooner than phosphamidon.

**Table 6.—Control of azalea lace bug with granules applied to the soil. 1959.**

| Ounces of Technical Systemic | Live Lace Bugs After (Days) | | | |
|---|---|---|---|---|
| | 10 | 14 | 18 | 27 |
| Dimethoate 0.03 | 0 | 0 | 0 | 0 |
| .06 | 0 | 0 | 0 | 0 |
| Phosphamidon .03 | 201 | 29 | 62 | 0 |
| .06 | 37 | 7 | 2 | 0 |
| Untreated | 602 | 139[a] | — | 315 |

[a] Two shakings only.

Control of Rhododendron Lace Bug.—On August 25, 1959, *Rhododendron maximum* averaging 4½′ high received soil treatments of dimethoate granules at the rate of 1 and 1½ pounds of formulation to 18.8 sq. ft. under each plant. There were three plants per treatment, and four untreated ones. Three inches of soil were raked away from the plants to their circumference, the granules were then applied and drenched with water both before and twice after the soil was replaced. This procedure was followed to protect roaming cats and dogs. As far as could be determined no animals were poisoned, although some digging under the treated plants occurred. Counts of adult lace bugs made before treatments as for scale insects previously described indicated an average of 11 per 10 shakings. An evaluation of the treatments made on September 4 by the same method as used for azaleas showed that there were no live adults on the treated plants. In addition the undersurface of about 200 leaves as examined for live nymphs without finding any. Many dead nymphs dropped from the treated plants as the adults always did. The same observation was also made on andromedas and azaleas.

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# Development of a Synthetic Medium for Aseptic Rearing of Larvae of *Stomoxys calcitrans* (L.)[1]

Richard E. Gingrich

### Abstract

A synthetic medium was formulated for rearing larvae of *Stomoxys calcitrans* (L.) aseptically. All the components were chemically defined with the exception of an aqueous extract from baker's yeast, without which development did not proceed beyond the first instar. The per cent survival of the larvae reared on this medium was almost as high as that of larvae reared on a nonaseptic medium. However the development time was somewhat longer. No attempt was made to determine which of the diet constituents were essential, or their optimum levels.

The stable fly, *Stomoxys calcitrans* (L.), has long been recognized as an annoying pest of livestock and man the world over. Although it has never been proven to be the natural vector of any agent of human disease it does serve as an intermediate host for the filarial worm *Setaria cervi* of deer, and the tapeworm *Hymenolepis carioca* of chickens. Investigations on the life history, behavior, and control of the stable fly have been a result of this species' economic importance, and laboratory rearing has become an important part of these investigations.

Under natural conditions *Stomoxys* larvae develop in a variety of materials, all having in common the presence of moist organic matter undergoing active microbial fermentation. This condition clearly suggests the importance of microörganisms for producing the necessary nutrients required for growth and development either through the breakdown of existing materials or through the synthesis of new substances that the larvae require.

Doty (1937), Eagleson (1943), Campau *et al.* (1953), Champlain *et al.* (1954), and McGregor & Dreiss (1955) have all reported on various methods and techniques for nonaseptic rearing of *Stomoxys* larvae on artificial media. A technique similar to the Peet-Grady method for house flies (*Musca domestica* L.) is generally employed. However, for carrying on certain studies, the rearing of test animals under sterile conditions is often necessary. With *Stomoxys* larvae the problem appears to be essentially one of supplying the necessary nutrients, normally supplied by the microorganisms present in their natural habitat, in a form suitable for larval consumption and utilization. Successful rearing of insects on a particular diet can depend on other factors even when all the essential nutrients are provided. Evidence shows that environmental conditions such as the physical properties of the food, the availability and utilization of nutrients, and the presence and nature of various stimuli to induce feeding responses are all factors to be considered.

The basic medium developed for rearing stable fly larvae aseptically and the results obtained with it are described here.

Experimental Procedure.—Albritton (1955) has presented exhaustive tables dealing with the formulation of synthetic diets for several species of insects. Few impor-

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tant taxonomic differences concerning nutritional requirements have been noted among the various groups, therefore in the formulation of the *Stomoxys* diet for these investigations, all the basic elements shown to be essential for other insects were included.

Initial attempts to rear *Stomoxys* larvae on media containing vitamin-free casein as a protein source were unsuccessful and led to the formulation of new diets using amino acid mixtures in place of casein. Levels of the individual acids used were based on the study by Friend & Patton (1956) of the amino acid requirements for the onion maggot (*Hylemya antiqua* (Meig.)) and the diet for *Aedes aegypti* (L.) (Lea *et al.* 1956). The vitamin mixture contained all the known essential B-complex vitamins with the addition of choline, inositol, and *p*-aminobenzoic acid. The levels used were generally those listed for *A. aegypti* (Albritton 1955). Dextrose was used as the carbohydrate and cholesterol as the lipid. Wesson's salt mixture was added as a mineral source. Ribonucleic acid, uracil, and guanine were used in later diets in an attempt to increase larval growth and survival. A water-soluble extract from dried baker's yeast was used for the yeast supplement.

All eggs used in these experiments were taken from the stock colony where the females had deposited them on a moist sponge. After eggs were washed from the sponge into a plastic container, they were stored in water at 4° C. until used. Eggs not used within 4 days were discarded.

To obtain aseptic larvae the egg surfaces were sterilized by the following procedure: several hundred eggs were transferred with a standard medicine dropper from the storage container into a piece of glass tubing 10 mm. in diameter and 60 mm. long, which was covered at one end with nylon organdy. After the eggs were placed in the tube, the open end was also covered with organdy and held in place with string. About 12 inches of string were left remaining at the knot to facilitate transferring the tube from one solution to another. A 2.5% lysol solution was used as the disinfectant.

The tube containing the eggs was first suspended in lysol solution for 2½ minutes. Proper timing is important because less time reduces effectiveness of the disinfectant and too much time decreases egg hatch. Then the tube, by means of the attached string, was removed from the lysol container and submerged in a bottle of sterile distilled water. The water was gently swirled 5 minutes so that the eggs were thoroughly washed free of all disinfectant. By raising the tube above the water level of the wash solution, all water was drained out, thus causing the eggs to collect on the organdy cover at the bottom end of the tube. The tube was then placed in a sterile petri dish with only a short portion of string remaining within the dish. The top of the petri dish was raised enough to allow the string, holding the organdy cover in place, to be cut with a sterile scapel. The stiffness of the organdy caused it to fall open and the sterilized eggs were left exposed on its surface. In this position the eggs could be picked up for transferral to the vials of diet.

Transfer of sterile eggs to the vials was carried out under a hood containing a General Electric ultraviolet germicidal lamp. The inside of the hood had been previously washed with lysol disinfectant followed by a 30-minute exposure to the germicidal lamp. A Bunsen burner was also placed inside. Using a small wire spatula, one egg was transferred to each vial of diet. The wire and vial tops were flamed with each transfer. After all vials had been inoculated they were placed in trays and stored in the dark at 28° C.

All vials were examined daily under a microscope and the day of hatching recorded as zero day. All events in larval development and adult emergence were then recorded in time from the day of hatching.

Eggs not hatched within 5 days were discarded. Larvae dying within 2 days of hatching were omitted from the experiment because of the possibility that they had been poisoned by the lysol used on the eggs.

It was not necessary to test each vial separately for sterility, as previous work had shown that the medium would readily support microbial growth and any contamination could be seen on its surface. Any vial showing contamination was discarded and not included in the results.

PREPARATION OF THE MEDIUM.—All the amino acids (table 1) except glutamic acid, aspartic acid, tyrosine, and tryptophan were weighed in amounts required to make 1 liter of medium, and were ground together in a mortar. Enough tyrosine for 1 liter of medium was dissolved in 100 ml. of hot water to which 3 ml. of concentrated hydrochloric acid had been added. The tryptophan was dissolved in 100 ml. of hot water. Aspartic and glutamic acids were each dissolved in 65 ml. of warm water. The four solutions of amino acids were combined and the mixture of all remaining acids was then added.

The guanine and uracil solution was made by adding 75 ml. of hot water, containing 0.25 ml. of concentrated hydrochloric acid, to 30 mg. of guanine and 4 mg. of uracil and heating over a steam bath for 1 hour. Two grams of ribonucleic acid were dissolved in 70 ml. of water plus 20 ml. of 2 N sodium hydroxide. Two grams of Wesson's salt mixture were dissolved in 100 ml. of hot water. Fifteen grams of dextrose were dissolved in 25 ml. of hot water.

A stock suspension of cholesterol was made by dissolving 500 mg. in 20 ml. of hot 95% ethyl alcohol. This solu-

**Table 1.—Composition of amino acid mixture used in preparing the basic diet.**

| CONSTITUENT | MG. PER ML. |
|---|---|
| DL-Alanine, alpha | 1.09 |
| L-Arginine, free base | 1.67 |
| L-Aspartic acid | 0.49 |
| L-Cysteine HCL | 0.48 |
| L-Glutamic acid | 4.42 |
| Glycine | 1.75 |
| L-Histidine | 0.48 |
| L-Cystine | 1.00 |
| DL-Isoleucine | 1.26 |
| L-Leucine | 2.35 |
| L-Lysine HCl | 1.34 |
| DL-Methionine | 0.34 |
| DL-Phenylalanine | 2.00 |
| L-Proline | 1.68 |
| DL-Serine | 0.88 |
| L-Threonine | 1.00 |
| L-Tryptophan | 1.23 |
| L-Tyrosine | 0.62 |
| DL-Valine | 3.00 |
| L-Hydroxyproline | 1.00 |

**Table 2.—Composition of the vitamin mixture used in preparing the basic diet.**

| CONSTITUENT | μG. PER ML. |
|---|---|
| Biotin | 0.05 |
| $B_{12}$ | 0.04 |
| Calcium pantothenate | 30.0 |
| Choline Cl | 100.0 |
| Folic acid | 20.0 |
| Nicotinic acid | 20.0 |
| Pyridoxine HCl | 20.0 |
| Riboflavin | 20.0 |
| Thiamine HCl | 20.0 |
| Inositol | 40.0 |
| *p*-aminobenzoic acid | 2.0 |
| Niacinamide | 50.0 |

tion was slowly poured into 100 ml. of cold water to which 0.3 ml. of Tween 80 (polyoxyethylene sorbitan monooleate) had been added. The result was a milky white suspension, which was warmed until the volume was reduced to 60 ml. by evaporation, to remove as much ethyl alcohol as possible.

The vitamins, choline, inositol and *p*-aminobenzoic acid (table 2) were prepared separately. Riboflavin was dissolved in 0.02N warm acetic acid and folic acid in 20% ethyl alcohol. All remaining vitamins and growth factors were each dissolved in distilled water.

Yeast extract was prepared by slowly adding 75 grams of dried baker's yeast to 100 ml. of warm distilled water. The suspension of yeast was heated to boiling and then allowed to stand until the insoluble portion settled out. The supernatant was decanted and saved.

The final medium (table 3) was prepared as follows:

1. The vitamin solutions were poured into a liter beaker followed by the salt and amino acid solutions. Ribonucleic acid, guanine, and uracil solutions were added and the pH adjusted to 6.5 with 2 N sodium hydroxide.
2. Twenty grams of agar were added to the beaker and the mixture was autoclaved for 2 minutes at 15 pounds pressure to melt the agar.
3. After removing the mixture from the autoclave, 100 mg. of cholesterol, in suspension, and the dextrose solution were added.
4. Seventy-five ml. of yeast extract were added and the entire mixture was poured into a liter volumetric flask.
5. Distilled water was added to bring the final volume to 1 liter and the flask was placed in an oven at 80° C. to keep the agar melted.

**Table 3.—Composition of basic diet.**

| CONSTITUENT | MG. PER ML. | μG. PER ML. |
|---|---|---|
| Agar | 20.0 | |
| RNA | 2.0 | |
| Guanine | 0.03 | |
| Uracil | 0.004 | |
| Cholesterol | 0.1 | |
| Dextrose | 15.0 | |
| Wesson's salt mixture | 2.0 | |
| Amino acid mixture | 28.08 | |
| Vitamin mixture | | 322.09 |
| Yeast extract | 0.075 | |
| Distilled water 1000 ml. | | |
| NaOH—Amount necessary to bring medium to pH 6.5 | | |

**Table 4.—Comparison of larval growth, pupation, and emergence as adults when fed on (1) sterile basic diet lacking yeast, (2) sterile basic diet including yeast, and (3) nonaseptic control diet.**

| DIET | NO. OF LARVAE | 2ND INSTAR | | 3RD INSTAR | | PUPA | | ADULT | |
|---|---|---|---|---|---|---|---|---|---|
| | | N[a] | T[b] | N | T | N | T | N | T |
| 1. Basic diet without yeast | 21 | 0 | | | | | | | |
| 2. Basic diet with yeast | 25 | 23 | 2.1 | 20 | 11.0 | 18 | 21.3 | 18 | 27.6 |
| 3. Control medium | 25 | 23 | 1.8 | 22 | 5.2 | 22 | 8.7 | 22 | 14.2 |

[a] N—Number of insects surviving from previous stadium.
[b] T—Average time in days required for insect to reach that stadium.

6. Two-ml. amounts of complete medium were then dispensed into 1-dram shell vials, which were plugged with cotton, arranged in trays, and autoclaved at 15 pounds pressure for 30 minutes.
7. After the vials were removed from the autoclave, they were placed in a position so that a slant was formed on cooling.
8. The vials, after the medium had solidified, were each inoculated with one sterile egg. Forty replicas of the basic diet were prepared.

RESULTS.—Observations on larval development are given in table 4. Larvae hatching on Diet 1 penetrated the agar medium and otherwise appeared normal. However, they did not undergo ecdysis to the second instar. The average length of larval life was slightly less than 6 days. One specimen lived 14 days but died while still in the first instar.

Larvae hatching on Diet 2 reached the second instar in an average of 2.1 days. The third instar was reached in 11.0 days and pupation occurred in 21.3 days. In 27.6 days the adults emerged, making this the total time for development from egg to adult.

Diet 3, prepared according to the method used at Ohio State University for mass rearing stable flies under nonaseptic conditions, was used as a control. The second instar was reached in an average time of 1.8 days, the third in 5.2 days and pupation in 8.7 days. The adults emerged in 14.2 days from the date of hatching. This time very closely approximates that of development under natural conditions.

The number of insects surviving, on the basic diet containing yeast, compared favorably with those on the control diet. Of the 25 larvae hatching on this basic diet, 18 emerged as adults, while on the control diet 22 of the original 25 emerged.

TESTS FOR POSSIBLE GROWTH FACTORS.—Since *Stomoxys* larvae grew, pupated, and emerged as adults only after addition of yeast extract to the basic diet, it appeared that some essential growth factor was supplied by the yeast. Attempts were made to identify the growth factor by substituting various substances for the yeast.

A suspension of pure animal lard, added to the medium, failed to improve the growth rate. Likewise, the addition of ascorbic acid or glutathione did not supply the missing factor.

DISCUSSION.—Other investigators working on the nutrition of insect larvae have established a list of substances that are considered essential in diets for growth and development. Under natural conditions these substances are either already present in a utilizable form, synthesized

by the larvae, or produced by microorganisms living in association with the larvae. In the preparation of synthetic diets it is necessary for the worker to supply these nutrients or to present the proper conditions for their synthesis. In cases where the nutrients are supplied by microorganisms the task of producing a sterile diet is increased, since the investigator must supply all the nutrients and in a form directly utilizable by the larvae. In many cases it has not yet been possible to rear larvae without the addition of some unidentified substances which in most cases may be found in some portion of yeast.

For *Stomoxys* larvae a chemically defined diet was prepared that contained substances known to be required for sterile cultures of insects living under similar conditions. However, this diet proved to be unsatisfactory for growth because the first instar did not molt. Only on the addition of an aqueous extract from baker's yeast were the larvae able to develop to maturity. This condition was probably due to some water-soluble growth factor in the yeast that was not in the previous diet. The fact that the physical conditions of the medium were unchanged after the addition of yeast to the diet lessens the possibility that physical factors had any influence on this requirement.

However, even after the addition of yeast extract the growth rate was slower than under natural conditions. This fact could be explained by several possibilities: it could be that there was missing some factor that was not absolutely essential for growth but one that was required for normal growth; the larvae may have been able to synthesize it themselves or grow slowly without it; physical differences between the agar medium and a natural medium such as decaying vegetation or the fact that the aseptic larvae were reared individually could have affected the growth rate; genetic differences among the individuals tested undoubtedly were responsible for some variations. Also since the nutrients used were not necessarily at optimum levels, they may have been inadequate for supporting normal growth rate.

The objective of this work was to develop a synthetic medium suitable for rearing *Stomoxys* larvae, and no attempt was made to determine which of the nutrients were essential, or their optimum levels. Any attempts along this line would have to be preceded by development of a diet in which all the constituents were chemically defined. Since the successful diet here required yeast, attempts to determine the essential nutrients would be unreliable.

CONCLUSIONS.—A sterile synthetic diet was prepared on which *Stomoxys calcitrans* larvae could be successfully reared. However, the growth rate was slower than that of larvae on nonaseptic media. The components were chemically defined with the exception of a yeast extract, which apparently supplied some essential growth factor. Animal lard, ascorbic acid, and glutathione failed to supply this factor.

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# The Excretion of Dieldrin, DDT, and Heptachlor Epoxide in Milk of Dairy Cows Fed on Pastures Treated with Dieldrin, DDT, and Heptachlor[1]

NORMAN GANNON[2] and G. C. DECKER[2]

ABSTRACT

To determine the degree of milk contamination that might be expected when dairy cows were permitted to graze on an insecticide-treated pasture for varying lengths of time and at various intervals after treatment, a series of tests was initiated using dieldrin, DDT, and heptachlor applied at about twice the rate that might be considered normal dosage (0.5, 3, and 0.5 lb. per acre, respectively). When cows were permitted access to pastures immediately after treatment, the chemicals reached their maximum concentrations in the milk within 3 to 7 days (dieldrin 3 to 4 p.p.m.; DDT 7 to 8 p.p.m.; heptachlor as heptachlor epoxide 0.22 p.p.m.) and declined steadily thereafter.

Shepard *et al.* (1949) and Biddulph *et al.* (1950) demonstrated that DDT, when fed to cows, was stored in fat and secreted in milk. Bann *et al.* (1956) proved that dieldrin did likewise. Davidow & Radomski (1953) showed that heptachlor metabolized and was stored in the fat of dogs as heptachlor epoxide. Later, Davidow *et al.* (1953) found that cows fed heptachlor secreted heptachlor epoxide in their milk. The project discussed here was initiated to determine the degree of contamination of milk from dairy cows that had been permitted to graze on pastures treated with dieldrin, DDT, and heptachlor.

PROCEDURE.—Materials were applied at rates about twice those that might be considered normal dosage; *i.e.*, 0.5 lb. of dieldrin and heptachlor and 3 lb. of DDT

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[2] Illinois Natural History Survey, Urbana.

per acre. Application was made with a ground sprayer bearing a 12-foot boom. The dieldrin experiment was conducted in 1957 and the pasture was sprayed at 7:00 a.m. July 30. The other materials were studied the following year. The DDT was sprayed June 23, 1958, and the heptachlor July 2, 1958. Holstein or Guernsey cows, selected for approximate uniformity in weight, food consumption, condition, and milk production, were placed on the pastures at varying intervals and for varying lengths of time after treatment. The experimental animals were in the treated pastures about 18 hours each day. They were regularly confined in the barn from 1:00 to 5:00 p.m. and from 4:00 to 6:00 a.m. each day, at which time they received 12 to 15 pounds of alfalfa hay and 8 to 10 pounds of a grain-concentrate supplement per head.

Milk samples representative of the total production at a regular milking were collected at intervals in one-half gallon milk cartons lined with pliofilm bags and were stored at 0° F. until analysis.

Samples of the pasture vegetation, which was a good mixture of alfalfa, brome grass, and some bluegrass, were also collected at intervals after treatment and analyzed for the various insecticides.

METHODS OF ANALYSIS.—*Dieldrin.*—Milk samples of 300 grams were cleaned up and analyzed for dieldrin according to Shell Method Series 638/56 (1956), which is a modification of the method of O'Donnell *et al.* (1955).

*Heptachlor Epoxide.*—This material was determined by first extracting 300 grams of milk with colorimetric pentane, 75 grams at a time in centrifuge bottles, and then combining the extracts. The method of Fahey (1957) was used for further cleanup and analysis. Final determination of heptachlor epoxide was done by reacting it with a reagent (0.5 M ethanolamine and 0.5 M potassium hydroxide in butyl Cellosolve) designed originally by Polen & Silverman (1952) for the determination of heptachlor.

*DDT.*—Following the extraction of 75 grams of milk with n-hexane, DDT was separated from butterfat by chromatography on a sulfuric acid:celite column after the method of Davidow (1950). Analyses were done by the method of Pontoriero & Ginsburg (1953).

RESULTS AND DISCUSSION.—The residues of the various insecticides found on the pastures at intervals during the feeding period are shown in figure 1. Since heptachlor converts to heptachlor epoxide on plants, persistence curves for both materials resulting from the heptachlor treatment are shown. Typical of such residue dissipation curves, all residues were very high initially, dropped rapidly for a day or two, and then leveled off to follow a slow, constant decline.

In view of the very rapid initial loss that occurs with highly volatile compounds, it appears such a loss during the short interval between application and initial sampling may account for the comparatively low initial residue recorded for heptachlor.

As anticipated from previous feeding studies (Gannon *et al.* 1959a, 1959b), dieldrin showed a very high propensity for storage in fat and excretion in milk. As shown in table 1, when cows were permitted immediate access to a treated pasture, dieldrin appeared in the milk within 12 hours and rose very rapidly to a peak of 3.5 to 4 p.p.m. by the second or third day and then went into an abrupt decline as the residue on the pasture and therefore the

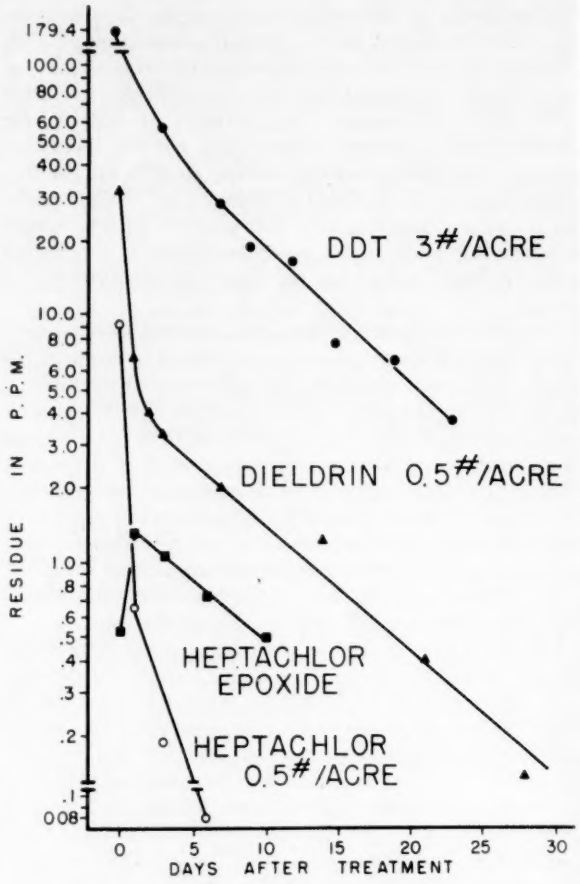


FIG. 1.—Residues on alfalfa-brome grass pasture at intervals after spraying.

dieldrin intake of the cow was reduced. The dieldrin content of milk from other cows turned into the pasture 3 and 7 days after treatment likewise tended to climb to a peak in 3 days and then to decline. However, when animals were kept out of the pasture until the 14th and 21st day, at which time the residue on the pasture had dropped to about 0.2 p.p.m., the dieldrin content in their milk rose much more slowly and did not reach a peak for about 3 weeks.

When animals were given only a short 3-day exposure to the treated pasture and then removed to a clean pasture, the dieldrin content of their milk showed the same typical rise but dropped off even more abruptly when the dieldrin intake was suspended and then tended to level off to a slow rate of decline typical of dieldrin in milk resulting from that stored in the animals' fat. In all cases the peak of the dieldrin content in the milk was more or less proportionate to the magnitude of the residue on the pasture at the time. The data seem to show clearly that at first the amount of dieldrin in the milk is determined largely by the daily rate of dieldrin intake in the diet. Later it is determined by the combined effect of daily intake and accumulated concentration in the fat. Finally, when dieldrin has been removed from the diet the slowly declining residues that persist in the milk for weeks must obviously be associated with dieldrin stored in the fat.

**Table 1.—Dieldrin residues in cows' milk when animals were permitted access to a treated pasture at various intervals after treatment and for varied periods of time.[a,b]**

| Days After Treatment | No. of Days After Treatment When Pasturing Started and Ended[c] | | | | | | | | | | Dieldrin on Pasture (p.p.m.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–56 | 3–59 | 7–63 | 14–70 | 21–77 | 0–1 | 0–2 | 0–3 | 3–6 | 7–10 | |
| | Days on Treated Pasture | | | | | | | | | | |
| | 56 | 56 | 56 | 56 | 56 | 1 | 2 | 3 | 3 | 3 | |
| 0 | X[d] | — | — | — | — | X | X | X | — | — | 31.8 |
| 0.5 | 0.46 | — | — | — | — | 0.44 | 0.27 | 0.31 | — | — | — |
| 1 | 2.24 | — | — | — | — | 1.36 | 2.33 | 1.85 | — | — | 6.9 |
| 2 | 4.11 | — | — | — | — | 1.90 | 2.53 | 3.72 | — | — | 4.1 |
| 3 | 3.04 | X | — | — | — | 2.29 | 3.24 | 3.39 | X | — | 3.4 |
| 4 | — | 0.46 | — | — | — | — | — | — | 1.46 | — | — |
| 5 | — | — | — | — | — | — | — | — | 1.75 | — | — |
| 6 | — | .88 | — | — | — | — | — | — | — | — | — |
| 7 | 1.95 | — | X | — | — | .84 | .86 | 1.12 | — | X | 2.04 |
| 8 | — | — | 0.17 | — | — | — | — | — | — | 0.17 | — |
| 9 | — | — | .49 | — | — | — | — | — | — | .37 | — |
| 10 | — | .67 | .46 | — | — | .70 | — | — | .19 | .65 | — |
| 14 | .75 | — | — | X | — | .29 | .44 | .57 | — | .18 | 1.25 |
| 17 | — | — | — | — | — | — | — | — | .22 | — | — |
| 21 | .72 | — | .31 | 0.19 | X | .26 | .66 | — | — | .13 | .41 |
| 24 | — | .40 | — | — | 0.11 | — | — | — | — | — | — |
| 28 | .58 | — | — | — | .16 | .27 | .42 | .35 | .16 | .12 | .14 |
| 31 | — | .35 | — | — | — | — | — | — | .15 | — | — |
| 35 | — | — | .21 | .18 | .17 | — | — | — | — | .12 | — |
| 42 | .37 | — | — | .22 | .20 | .33 | .39 | .35 | — | — | — |
| 46 | — | .33 | — | — | — | — | — | — | .14 | — | — |
| 49 | — | — | .28 | — | .15 | — | — | — | — | .08 | — |
| 56 | .38 | — | — | .10 | — | .16 | .28 | .18 | — | — | — |
| 59 | — | .29 | — | — | — | — | — | — | .12 | — | — |
| 63 | — | — | .14 | — | .11 | — | — | — | — | .09 | — |
| 70 | — | — | — | .08 | — | — | — | — | — | — | — |
| 77 | — | — | — | — | .05 | — | — | — | — | — | — |

[a] Sprayed with 0.5 lb. dieldrin emulsion/acre.
[b] Sensitivity—0.01 p.p.m. in milk.
[c] One cow per exposure time.
[d] Cow placed on pasture.

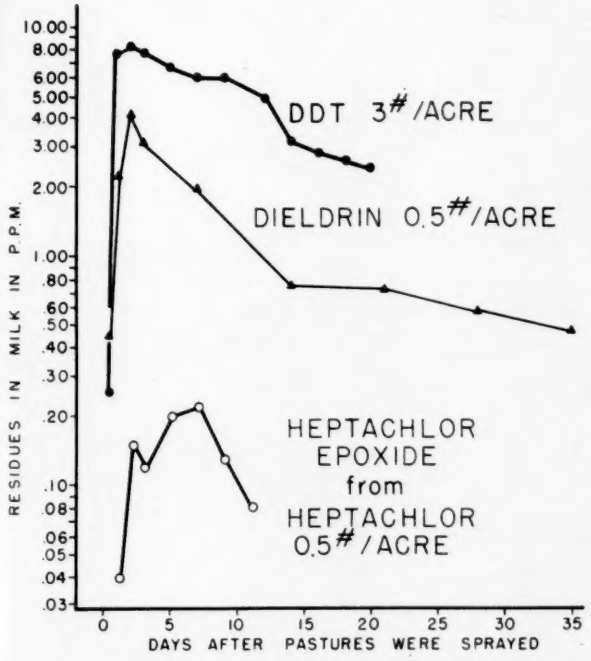


FIG. 2.—DDT, dieldrin, and heptachlor epoxide found in cows' milk at intervals when cows had access to a pasture immediately after treatment and thereafter.

The hazards that accrue when animals are permitted immediate access to a treated pasture are clearly illustrated in figure 2. It seems apparent that insofar as dieldrin in milk is concerned, much of the damage was done in the first 2 or 3 days. After that it did not make a great deal of difference whether the cow had continued access to the pasture or not. Fifty days after treatment a cow that had access to the pasture for the first 3 days only had nearly twice as much dieldrin in her milk as did another cow kept out of the pasture for 14 days and then pastured therein for the next 36 days.

The pattern of DDT excretion in milk follows that of dieldrin quite closely. A peak is reached in about 3 days, but because DDT residues are higher, more persistent and remain high longer, the dropoff is not so pronounced (table 2 and fig. 3).

The peculiar pattern displayed by heptachlor (table 3 and fig. 3) seems to reflect the low propensity for storage of heptachlor (Gannon *et al.* 1959b) and the suspected higher pattern of its decomposition product, heptachlor epoxide, which may more closely resemble dieldrin.

Since the tolerances for these materials in milk is zero, these data, particularly those for dieldrin and DDT, show quite clearly the need for excluding dairy cows from treated pasture for long periods after treatment. With the dieldrin treatment, while contamination was measurable in cows exposed as late as 3 weeks after treatment, more contamination occurred during the first 2 or 3 days than

**Table 2.—DDT residues in cows' milk when animals were permitted access to a treated pasture at various intervals after treatment and for varied periods of time.[a,b]**

| Days After Treatment | No. of Days After Treatment When Pasturing Started and Ended | | | | | DDT on Pasture (P.P.M.) |
|---|---|---|---|---|---|---|
| | 0–20[c] | 0–4[c] | 3–20[d] | 7–20[d] | 14–20[d] | |
| | Days on Treated Pasture | | | | | |
| | 20 | 4 | 17 | 13 | 6 | |
| 0 | X[e] | X | — | — | — | 179.4 |
| 0.5 | 0.26 | 0.24 | — | — | — | — |
| 1 | 7.55 | 4.16 | — | — | — | 95.0 |
| 2 | 8.14 | 4.89 | — | — | — | — |
| 3 | 7.62 | 6.64 | X | — | — | 58.0 |
| 3.5 | — | — | 1.73 | — | — | — |
| 4 | — | — | 5.61 | — | — | — |
| 5 | 6.72 | 3.82 | 5.53 | — | — | — |
| 6 | — | — | 6.91 | — | — | — |
| 7 | 5.97 | 1.50 | — | X | — | 28.5 |
| 7.5 | — | — | — | 1.80 | — | — |
| 8 | — | — | 4.72 | 3.33 | — | — |
| 9 | 5.86 | 1.63 | 5.44 | 4.01 | — | 19.0 |
| 12 | 4.79 | 1.25 | 3.12 | 3.36 | — | 16.6 |
| 14 | 3.13 | 1.33 | 3.35 | 1.96 | X | — |
| 14.5 | — | — | — | — | 0.71 | — |
| 15 | — | — | — | — | 1.16 | 7.8 |
| 16 | 2.82 | 1.19 | 2.80 | 1.82 | 1.12 | — |
| 17 | — | — | — | — | 1.60 | — |
| 18 | 2.6 | 1.66 | 2.03 | 1.27 | — | — |
| 19 | — | — | — | — | 1.05 | 6.7 |
| 20 | 2.35 | 1.05 | 2.25 | 1.27 | 1.45 | — |
| 23 | — | — | — | — | — | 3.8 |

[a] Sprayed with 3 lb. DDT emulsion/acre.
[b] Sensitivity—0.04 p.p.m. in milk.
[c] Average of two cows.
[d] One cow.
[e] Cow placed on pasture

**Table 3.—Heptachlor epoxide residues in cows' milk when animals were permitted access to a (heptachlor) treated pasture at various intervals after treatment and for varied periods of time.[a,b]**

| Days After Treatment | No. of Days After Treatment When Pasturing Started and Ended | | P.P.M. on Pasture | | Total Toxicant (P.P.M.) |
|---|---|---|---|---|---|
| | 0–12[c] | 5–12[d] | Heptachlor | Epoxide | |
| 0 | X[e] | — | 9.20 | 0.53 | 9.73 |
| 1 | 0.04 | — | .66 | 1.37 | 2.03 |
| 2 | .15 | — | — | — | — |
| 3 | .12 | — | .19 | 1.06 | 1.25 |
| 4 | — | — | — | — | — |
| 5 | .20 | X | — | — | — |
| 6 | — | 0.08 | .08 | .74 | .82 |
| 7 | .22 | .09 | — | — | — |
| 9 | .13 | — | — | — | — |
| 10 | — | .06 | .05 | .51 | .56 |
| 11 | .08 | — | — | — | — |
| 12 | — | .02 | — | — | — |

[a] Sprayed with 0.5 lb. heptachlor emulsion/acre.
[b] Sensitivity—0.01 p.p.m. in milk.
[c] Average of four cows.
[d] One cow.
[e] Cow placed on pasture.

in all the rest of the time put together. DDT, being more persistent, although less readily absorbed than dieldrin, caused fairly high levels to appear in milk when cows were exposed as late as 2 weeks after treatment. Cows exposed to the heptachlor treatment immediately after application showed only 0.22 p.p.m. of epoxide at most in their milk. This compares with 4.11 p.p.m. dieldrin at the same dosage and 8.14 p.p.m. DDT at 6 times the dosage (fig. 3). The level of contamination caused by exposing cows to heptachlor immediately after spraying is comparable to that obtained by pasturing cows on dieldrin 2 to 3 weeks after spraying.

Throughout the experiments all the cows remained in good health and showed no evidence of being in any way affected adversely by the treatments.

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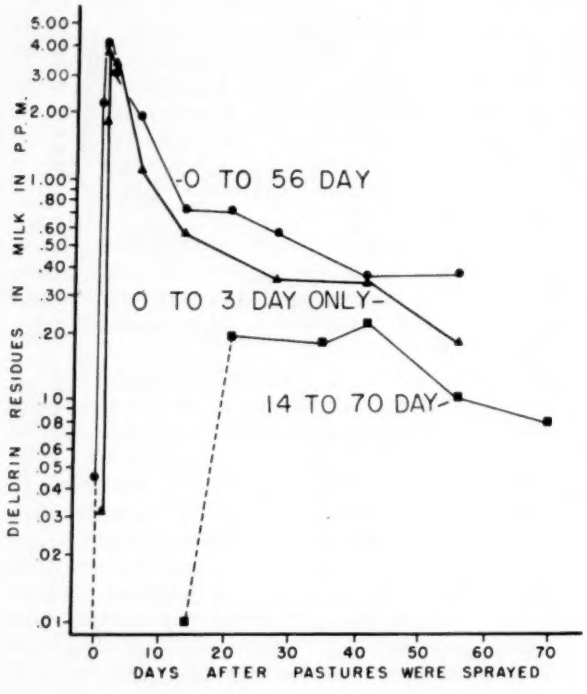


FIG. 3.—Dieldrin residues in milk of three cows when cows had access to a dieldrin-sprayed pasture at the times and intervals indicated.

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# Use of Insects for Their Own Destruction[1]

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ABSTRACT

In considering ways of controlling or eradicating insect populations, the importance of a fuller understanding of the population density in an area is stressed. Knowledge of the rate of increase of population from one generation to the next is basic to an understanding of the degree of control that is required to hold insects to noneconomic levels. Theoretical calculations are presented to show that a low-level mortality that is constant and superimposed on mortality produced by normal environmental resistance can in time lead to a greatly reduced population. The importance of applying control measures against the total population, rather than against segments of the population is pointed out.

Four ways of using an insect species to destroy its own kind are: (1) The release of insects made sexually sterile by gamma radiation, the only one that has already been employed successfully; (2) the use of chemicals that produce sexual sterility in the natural population of insects. The advantage of the desired type of chemical sterilants over chemicals that kill the insects is shown by theoretical trends in the population of a species subjected to the two types of control; (3) the release of adults infected with pathogens that would destroy larval progeny by transmitting the disease to other adults and contaminating the environment where the insect reproduces and develops; (4) the development and release of insect strains that carry deficient genetic characteristics. Such genetic deficiencies should be of a type that would not prevent rearing of the insect under controlled conditions or seriously interfere with the ability of the released males to compete with normal males in mating. Such genetic lethal factors would have to be expressed in the immature progeny or become lethal in the adult during hibernation.

The success of the sterile-male method of eradicating the screw-worm (*Callitroga hominivorax* (Cqrl.)) from Curacao and the southeastern United States has suggested the possibilities of utilizing sexually sterile insects for controlling or eradicating other species. Also, I have given considerable thought to other ways of employing an insect to destroy its own kind. Four ways of utilizing the autocidal principle of insect control are proposed for exploration in further research:

(1) Release of males that have been made sexually sterile by gamma irradiation or other means.

(2) Development of chemicals or other means of inducing sexual sterility in the natural population of an insect.

(3) Development and release of strains of an insect that possesses inferior or lethal genetic characteristics.

(4) Release of insects to spread pathogenic organisms.

Considerable research wil be necessary to determine if these approaches will be effective and practical for controlling various insects. From a theoretical standpoint, all the methods could be highly effective, and I think they are worthy of exploration. I might add that I have enough confidence in research by competent determined scientists to believe that seemingly impossible obstacles can be overcome if the basic principles are sound.

The basic principle of releasing sexually sterile males to control insects and other organisms has been proved sound. Several publications relate to this subject (Bushland & Hopkins 1951, 1953, Baumhover *et al.* 1955, Knipling 1955, Lindquist 1955, Knipling 1959). It therefore seems reasonable to assume that other means of using insects to control their own kind could also be developed. One point in favor of these methods is the almost certain premise that release of males carrying some lethal factor will be the most efficient procedure that can be devised for locating and destroying females. It therefore seems worthwhile to consider conducting research on any potentially feasible way of utilizing an insect to destroy its own kind.

To develop this concept it will be necessary to give much more attention to nutrition and culture methods for insects, dispersal and mating habits, population dynamics, and factors influencing the rate of development. A basic consideration of the population dynamics of an insect species is so important in applying the autocidal approach to insect control that I should like to present some of my views on this matter.

[1] Presented at the meeting of the Eastern Branch, Entomological Society of America on October 30, 1959, at Atlantic City, New Jersey, under the title "Novel Approaches to Insect Control." Accepted for publication November 30, 1959.

**Table 1.—Estimated boll weevil population and square infestation trends starting with winter-surviving population of 20 per acre under low, moderate, and high biotic increase rates per generation.**

| Fruiting Period[a] | Number of Squares/Acre | Low Increase (2.5-Fold) | | Moderate Increase (5-Fold) | | High Increase (7.5-Fold) | |
|---|---|---|---|---|---|---|---|
| | | Number of Weevils/Acre | % Squares Infested | Number of Weevils/Acre | % Squares Infested | Number of Weevils/Acre | % Squares Infested |
| First | 100,000 | 20 | 2.5 | 20 | 2.5 | 20 | 2.5 |
| Second | 200,000 | 50 | 3.7 | 100 | 6.3 | 150 | 9.5 |
| Third | 100,000 | 125 | 15.7 | 500 | 63.0 | 1,125[b] | 100.0 |

[a] A period of 3 weeks is considered a fruiting period, which is about the lengthof time required for one generation of the insect.
[b] This number could furnish enough migrants to infest all the squares on about another half acre.

Population Dynamics in Relation to Insect Control.—Before we can utilize insects to destroy themselves, we must have information on the total number in the environment at the different levels in their abundance cycle. We need to know the norm and extremes of the rate of increase from one generation to the next and also the effect on the total population when various levels of control are applied over a period of several generations.

It is my opinion that the overall population of species can be drastically reduced in time if a new hazard to its survival is applied uniformly and constantly even at a relatively low level. The level of control that will cause a significant decline can of course be expected to vary with the species and with ecological conditions.

I have done considerable theorizing on the probable rates of increase of different insect species from one generation to the next under normal conditions, and also on the rate of increase when new hazards to survival are introduced over and above usual environmental hazards. I have given considerable attention to the boll weevil (*Anthonomus grandis* Boh.) in such studies, the population dynamics of which I believe would be fairly representative of those of a considerable number of our major pests. Table 1 gives the estimated rate of increase of this insect starting from a low level during seasons that would be unfavorable, average, and favorable for the boll wevil. On the basis of available information on the feeding and oviposition habits of this insect and the fruiting behavior of the cotton plant producing moderate yields of cotton, the percentage of cotton squares that would be punctured at the different population levels has also been estimated. In these calculations I have assumed that each boll weevil will puncture six squares per day. Cotton yielding about 1¼ bales per acre could be expected to produce about 400,000 squares per acre in one season.

If we accept the estimate of a fivefold increase rate per generation as representative for many insect species, we can speculate on the effect on the total population over a period of several generations if we introduce new hazards that destroy various percentages of the insect above those resulting from the normal hazards. Assuming that the hazards during the winter are reasonably constant we can project the effects of such hazards into the subsequent season. As examples of new hazards we might assume that the rate of increase is reduced each generation by introducing a new parasite, by developing and planting a resistant variety, by releasing sterile males, or by any other procedure that reduces survival at a uniform and constant level.

In order to appraise the long-range effect that is possible by introducing such new hazards, I have made some theoretical calculations that are presented in table 2. These calculations are based on my estimates of the population dynamics of the boll weevil but are presented in efforts to establish principles applicable to insects in general.

I wish to emphasize that the figures are theoretical. It is recognized that the number of insects in a given area will vary from year to year and the rate of increase will vary from generation to generation. My purpose in presenting the figures is to suggest what might be achieved under average conditions if a new low-level control measure were applied and superimposed on other control factors for an extended period of time. Although theoretical, these figures may be fairly realistic. We know that an upset in the balance between destructive and beneficial insects through the use of insecticides can cause outbreaks of the host under circumstances when rates of parasitism are normally not above 50%. Conversely, newly introduced insects often develop high populations for several years but subside to a much lower population level as new parasites or predators become established, even though the rate of parasitism may not exceed 25% to 50%.

In the example given in table 2, several assumptions are made: (1) that the insect has attained stability in its environment and is subjected to hazards in development that are reasonably constant from year to year; (2) that the normal increase rate averages fivefold from one generation to the next; and (3) that 8% of the hibernating generation survives the winter and enters the field; and (4) that the growers in the area use insecticides that destroy 90% of the weevils that survive hibernation.

**Table 2.—Theoretical effect on the total population of boll weevils when subjected to hazards that raise the mortality by 50% above normal on the assumption of 8% survival of hibernating insects and 90% destruction of hibernating survivors by insecticides. Number of insects in 50,000 acres of cotton.**

| Generation | Under Normal Conditions | Following Introduction of New Hazards That Produce 50% Kill Above Normal | | |
|---|---|---|---|---|
| | | First Year | Second Year | Third Year |
| Survivors | 1,000,000 | 1,000,000 | 125,000 | 15,000 |
| First | 5,000,000 | 2,500,000 | 312,000 | 38,000 |
| Second | 25,000,000 | 6,250,000 | 780,000 | 95,000 |
| Third | 125,000,000 | 15,625,000 | 1,950,000 | 237,000 |

Although hypothetical, I believe that these assumptions and the insect population trends for an average community are not too unrealistic for the boll weevils on the basis of studies that I have made utilizing information from published reports of various investigators and information obtained through inquiries from a number of authorities. I have concluded that 200 overwintered boll weevils per acre of cotton is a fairly normal level in the spring, also that 90% mortality of overwintered boll weevils can be expected in a community practicing early-season control. Thus, 20 insects per acre is suggested as the average population level in the spring in a cotton-growing community practicing early-season boll weevil control. An assumed population level of about 20 per acre would also be fairly realistic in northern fringe survival areas, where mortality due to cold weather is generally high.

The theoretical population trends under conditions of assumed normal hazards and cotton-growing practices, as compared with the trends when the rate of increase is further reduced by 50%, are very interesting and may be highly significant in their implications.

It is recognized that the population trend will not continue downward indefinitely, nor is it likely even to approach the level indicated for the second or third seasons. Various population density factors will no doubt come into play and permit a higher percentage survival as the result of the overall decline. However, I do believe that under the circumstances cited, the average population would become stabilized at a level considerably below the former level because of the introduction of a new hazard that is constant in its effect on the total population. A new constant hazard that destroys about 50% of the insects in excess of those destroyed by the normal environmental resistance factors could in a few years have such a drastic effect on the population that the insect would become of minor economic significance. I would like to urge research on various insect species to determine what effect would be produced on total populations in isolated areas when subjected to such low levels of control. It would be desirable, of course, to achieve higher levels of control if possible. In this connection it is interesting to note the relative population trends of an insect subjected to high levels of control on the entire population as compared with the population trend when the level of control is near perfect on all but a small percentage of the host plants in a community.

Table 3 shows the assumed normal trend of an insect population starting from a low level in the absence of control; 100% control on 90% of the acreage each generation; and 90% control each generation on 100% of the acreage in a community. A casual consideration of the relative effect on population trends produced by the two types of control programs would suggest that the results would be essentially the same. However, on the assumption that population increases are largely confined to the original infested fields until the insects are forced to disperse because of lack of food or to seek hibernation quarters, the total population in the community obtaining moderately high control on all acreage could be reduced a hundred times as much as in the community where 10% of the acreage received no control but 100% control is achieved on the remaining 90% of the acreage.

**Table 3.—Theoretical population trend of an insect species in a community subjected to: (1) no control, (2) treatments that destroy all insects on 90% of acreage each generation with no control on remaining 10%, (3) community-wide treatments that destroy 90% of the insects each generation on all the acreage.**

| Generation | Number of Insects in 50,000 Acres of Crop | | |
|---|---|---|---|
| | Condition 1 (no control) | Condition 2 | Condition 3 |
| Winter survivors | 1,000,000 | 1,000,000 | 1,000,000 |
| $F_1$ | 5,000,000 | 500,000 | 500,000 |
| $F_2$ | 25,000,000 | 2,500,000 | 250,000 |
| $F_3$ | 125,000,000 | 12,500,000 | 125,000 |

Although several methods of insect control such as chemicals, resistant varieties, or cultural control procedures might be used to obtain the desired results, I believe that insects themselves might provide the most practical and efficient way to obtain maximum results if ways to achieve autocidal control can be developed.

The four ways of utilizing the autocidal principle of insect control suggested herein follow:

(1) RELEASE OF STERILE MALES FOR INSECT CONTROL.—The production and release of insects made sterile by gamma radiation might be developed to control numerous other important species in addition to the screwworm. The method has shown sufficient promise, based on laboratory tests, to warrant pilot eradication experiments against certain tropical fruit flies. The fruit fly laboratory at Honolulu, Hawaii, is making plans for eradication experiments against the oriental fruit fly (*Dacus dorsalis* Hendel) and melon fruit flies on the island of Rota in the Pacific in cooperation with the U. S. Navy and Trust Territory. The use of sterile males of the Mexican fruit fly (*Anastrepha ludens* (Loew)) to prevent establishment of invading insects from Mexico also offers great promise. In this connection it should be pointed out that fruit flies have multiple mating habits.

Excessive radiation damage which reduces the ability of the males to compete with normal males may prove to be the most significant obstacle to the practical application of this method of insect control. If radiation or other suitable ways of inducing sexual sterility can be developed, and if it is feasible to rear large numbers of a particular insect at a reasonable cost, I believe there will be many situations where the production and release of sterile males could provide a practical method of control. Workers have a tendency to be discouraged from considering this approach because of the large number of insects assumed to be present in the natural population. It is my opinion that the rearing of adequate numbers of insects for sterilization and release will not be the most formidable obstacle. If we had effective methods of producing sterility without serious damage to the males, we could for many of our major pests first reduce the natural population to a very low level through the use of chemicals or cultural practices and then release enough sexually vigorous sterile males to reduce the surviving population to the vanishing point. Once the natural population is eliminated, it might then be possible to keep the insect from becoming re-established through continuous releases of a

relatively few sterile insects, even in areas subjected to repeated reinfestation by natural dispersion or by accidental reintroductions. The cost of maintaining control by organized and continuous release programs throughout the favorable season might be only a fraction of the initial eradication cost and could be well below the annual cost of control by current methods.

In line with the earlier discussion on population trends of an insect subjected to low-level control procedures, the use of sterile insects might be the most effective way to achieve such control. A chemical control procedure that destroys 50% of the insect in each generation above that achieved by usual insect control practices, or the planting of a resistant variety that would result in a similar reduction might have cumulative effects that would be highly significant. This supposition is indicated in table 2. However, the maximum effects would be the same each generation and each year. The release of sterile males in sufficient numbers to reduce the biotic increase rate by 50% each generation during the first year, if repeated the second year using the same number of insects, would be much more effective. As the natural population declines, the release of sterile males at a constant level would provide such a high ratio of sterile to fertile insects that eradication might be expected. The release of just enough sterile insects to effect 50% reduction in the normal increase rate each generation during the first season and then the continuance of such rate of release each season might be a more practical and economical way to achieve insect eradication than to attempt to release enough sterile insects to dominate the population by 90% or more during the first generation.

Research is urgently needed to develop means of causing sterility in insects without affecting their sexual vigor. Exposure to gamma rays is one way, but much research needs to be done on various stages of insects and under different conditions to determine the best procedure to follow. Other methods of inducing sterility need to be investigated; perhaps chemical sterilants for use under controlled conditions might be developed. Mitlin *et al.* (1957) and Mitlin & Baroody (1958) have shown that a number of chemicals will prevent ovarian development in the house fly (*Musca domestica* L). The production of sterile hybrids as a result of crossing two related species or races might be possible for some insects without changing their mating behavior or ability to find the females of the natural population. The possibility of this approach for tsetse fly (*Glossina* spp.) control was suggested by Vanderplank (1947) and for the gypsy moth (*Porthetria dispar* (L.)) by Downes (1959).

(2) POTENTIAL VALUE OF A CHEMICAL CAUSING SEXUAL STERILITY IN NATURAL POPULATIONS OF INSECTS.—Preventive programs with chemicals now depend on immediate kill of enough of the insects to prevent increase of the population to damaging levels. Let us consider the theoretical possibilities of a chemical that would cause sexual sterility in all exposed individuals without otherwise affecting their mating vigor. I do not know whether it is possible to find such a chemical, but if such could be developed, we would have available a new approach to insect control that could be truly revolutionary.

The theoretical possibilities of this approach to insect control are shown in table 4. The same general assumptions discussed on normal population trends have been made. If the normal rate of increase from the winter survivors is fivefold per generation, the population reduction by a chemical that destroys 90% of the insects each generation by killing is much more gradual than that caused by a chemical that would produce sexual sterility. With such a chemical it would probably be easier to eradicate certain insects than to obtain economic control with current insecticides that depend on immediate kill. The reason for the greater effect of the sterilizing chemical is readily apparent. Insects that are made sexually sterile cannot contribute to further increases in the population. In turn, however, if 90% are sterile, they can theoretically reduce the biotic potential of the 10% survivors by 90%.

**Table 4.—Theoretical population trends of an insect species subjected to no treatment and to a treatment that affects 90% of the population by killing or sexual sterilization.**

| GENERATION | NO TREATMENT | TREATMENT THAT KILLS | TREATMENT THAT INDUCES STERILITY |
|---|---|---|---|
| Parent | 1,000,000 | 1,000,000 | 1,000,000 |
| $F_1$ | 5,000,000 | 500,000 | 50,000 |
| $F_2$ | 25,000,000 | 250,000 | 2,500 |
| $F_3$ | 125,000,000 | 125,000 | 125 |

In my opinion the potential advantages of sexual sterilants over killing agents for use in insect control are so great that intensive research to find such chemicals is warranted. Scientists are making progress in developing chemicals that cause sexual sterility in higher animals. I feel confident that chemicals having such action could also be found for insect control and eradication, if intensive basic and applied programs of research were initiated on a variety of major insect pests.

(3) DEVELOPMENT AND RELEASE OF STRAINS OF INSECTS THAT POSSESS GENETIC LETHAL FACTORS.—Through variations in the genetic makeup of insects nature permits them to become better adapted to survival in a changing environment. Environmental resistance factors through the ages have improved the adaptability of species by eliminating the least adaptable individuals in a population. Man has taken advantage of genetic variations in organisms and through selection has produced greatly improved plant and animal species with characteristics that increase their chances of surviving natural hazards.

To my knowledge man has not set out to produce organisms that are inferior. What are the opportunities for deliberately producing strains of destructive insects that are inferior in one or a number of ways and thus are more susceptible to normal environmental resistant factors? Personally, I believe this approach is possible. It might be far easier, through selection, to produce inferior organisms than it is to produce superior organisms.

If the development of inferior insect strains is feasible, how can we utilize them to control insects? I again propose their production and sustained release in sufficient numbers during favorable periods to dominate the natural population and in this way transfer the undesirable traits into the natural population in sufficient frequency to lead to its destruction owing to environmental resistance factors which eventually take effect.

It is the general concept of biologists that nature will quickly reduce the frequency or eliminate undesirable genes from an insect population and naturally occurring or even introduced undesirable mutant genes will have little effect on the population as a whole. However, I believe the concept of dominating the natural population through initial and sustained releases of large numbers of inferior organisms has not been considered. It seems entirely feasible through selection of undesirable genetic traits to develop strains of an insect that can be reared under controlled conditions and in the adult stage will survive long enough when released in the natural environment to compete effectively with the native population in mating and thus transmit the undesirable traits. If so, then the problem is to produce and release enough insects to dominate the natural population to the degree required. Undoubtedly there are many types of genetic defects in an insect species that could be considered.

The genetic deficiencies should not interfere to any great degree with the production and survival of the insect under controlled laboratory conditions or reduce survival or ability of the released males to compete with the males in the natural population. However, the genetically deficient genes must be of such nature that they will prevent survival of the progeny in nature during some period in the seasonal cycle. The following types of genetic deficiencies might meet these requirements:

*Inability to Diapause.*—If a strain of a normally diapausing insect could be developed that could not diapause, such a trait would be of no disadvantage in rearing under controlled conditions; in fact, it could be a distinct advantage. Moreover, such a trait would not limit survival of the insect when released during the regular period of reproduction in the growing season. However, all progeny carrying such deficiency would fail to survive the winter because they could not hibernate. Biologists have already shown that the diapause characteristic in certain insects is genetically controlled, in part at least. Thus, this deficiency would seem to be a desirable lethal character.

*Inability to Fly.*—Certain insects after reaching their host plants are capable of feeding and reproducing during the growing season without flying. However, ability to fly would be vital to survival both before and after hibernation. The boll weevil is such an insect. It might be possible to develop strains of the boll weevil that would be incapable of flying. Perhaps several genes, independent in action, could be found and bred into strains that could not fly. Inability to fly would not interfere with production of the insect under controlled conditions. When adequately dispersed, such deficiency would probably not reduce the ability of males released in cotton fields to compete with normal males in mating with females. However, such defect would lead to death of most or all individuals so affected during the fall, winter, or spring because they could not fly to hibernation areas or to cotton fields in the spring.

*Deficiencies in Immature Stages.*—It would seem possible to develop strains of insects with deficiencies in immature stages which would not be serious handicaps to survival in artificial media in the laboratory, but might be lethal to individuals attempting to develop on their normal host. The inability of an insect to glue its eggs on a plant or the absence of vital spines on larvae and deformed mouth parts in larvae are examples of deficiencies that might interfere with development of the insect in nature but would not seriously affect their survival in the laboratory. Adults of such strains might survive in their natural environment and be fully competitive in mating with the natural population.

If a dominant or several independent recessive characteristics of the type suggested could be found and fixed in an insect strain, such traits might be more useful for control or eradication than the induced damage to genetic material caused by exposure to gamma irradiation. As previously stated, gamma irradiation has a serious effect on the longevity and mating vigor of certain insects. Among multiple mating species, sperms from irradiated males apparently do not compete fully with sperms from normal males. On the basis of current knowledge, radiation damage might be the most important obstacle to the use of this sterility approach. One dominant or three or more recessive lethal characteristics independent in action might be equally as effective as induced sterility among monogamous insects and be much more effective among polygamous species.

(4) RELEASE OF INSECTS TO SPREAD PATHOGENIC ORGANISMS.—Insect pathology is a growing phase of entomology, and the use of pathogens to control destructive insects is receiving more and more study. In view of their importance as natural control factors for numerous major pests, there is every reason to believe that through research and experience diseases of insects will prove useful for the control of many species. How to apply insect pathogens to produce the desired effect is one of the major problems. I wish to propose for consideration that research be undertaken to determine if insects can be used to disseminate diseases among their own kind.

Several factors seem to favor this approach:

(1) Certain pathogens are nonvirulent when present in or on the adults but are highly virulent to the larvae.

(2) For some disease organisms the amount or number necessary to cause infection in the early larval stages is extremely small.

(3) The adult of any given species in seeking a mate or oviposition site will naturally find the environment where the disease is most likely to have the best opportunity to spread.

(4) If spread of an organism to adults in the natural population can be achieved through mating by releasing infected adults, it seems safe to assume that the insects released will be capable of finding individuals of their own kind more effectively than can be done by any other method.

At my suggestion, C. G. Thompson, of the Insect Pathology Laboratory of the Entomology Research Division, has undertaken investigations that have already shown results that justify further study.

There seem to be two ways in which adult insects might be used to spread an organism. Certain viruses are much more pathogenic in the early larval instars than in the late instars. According to Dr. Thompson, there are indications that larvae infected late in the larval stage will transmit the disease in lethal amounts through the adult to newly hatched larvae of the next generation. The other possibility is to contaminate the adults directly with virus ma-

terial so that the eggs or plants on which eggs are deposited will become sufficiently contaminated to infect newly hatched larvae.

Both methods seem to warrant study. If it could be shown that adults infected systemically or by external contamination would cause 100% infection early in the larval stage, the released insects themselves would not damage the crop or cause an increase in the overall population. If in turn, however, the contaminated insects that are released caused a high incidence of infection among the progeny of the natural population, the overall population would be reduced. There are several ways this might occur: (1) by spreading the disease to adult females through mating with infected and released males, thus exposing the progeny to infection; (2) by contaminating plants where the released insects rest, oviposit, or feed which in turn could lead to infection of larvae from normal uninfected adults in the natural population; and (3) by increasing the amount of virus in the environment if larval progeny of released insects become infected.

As with other ways to utilize insects to destroy their own kind, the method may be practical only when the natural population is at a low level so that the number needed to dominate it will not be too great. For most species this might be a requirement in order for it to be economically feasible to rear and release the required number of insects. In some instances, however, the release of a relatively few infected adults might be the most effective and practical way to introduce a new disease into a high natural population of an insect or cause an epizootic of a common disease before it would normally occur.

The spread of insect pathogens by deliberately releasing infected adults seems to offer unusual possibilities, especially if it can be shown that the released insects could spread the disease to adults in the natural population through mating. If mating of an infected male to an uninfected female would assure fatal disease among all the larval progeny, the final effect on the total population could be greater than the release of gamma-irradiated sterile males even among insects that have single mating habits. The matings would not only destroy the reproductive potential of females actually mated to released infected males, but the diseased larval progeny and the infected adults might cause further spread of the disease by contaminating the plants.

The greatest advantage over the sterile-male method could be among multiple-mating species. For example, among these species, such as tropical fruit flies and mosquitoes, it is apparently necessary to release up to three sterile males to compete with one fertile unirradiated male. On the same basis, a disease-contaminated male could theoretically destroy the reproductive capacity of several females if the organism is transmitted to all females with which it mates. In applying the male-sterility method among polygamous species, it might be necessary to release 900 gamma-irradiated sterile insects to reduce the biotic potential of a natural population of 100 insects per acre by 75%. To achieve the same reduction with disease-contaminated insects it would be necessary to release only about 55 insects if mating and infection of mates take place an average of three times.

Extensive investigations with different insects and pathogenic organisms under various ecological situations might be required to demonstrate practical control by this method. However, on theoretical grounds, this approach offers unusual opportunities. As with other techniques involving the release of insects, ways to rear insects in large numbers and effective dispersal procedures are prerequisites to success of the method. It is also likely that success would depend on application of the method to the entire natural populations in large areas.

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# Economic Importance of the Chicken Body Louse[1]

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ABSTRACT

Four pullets and a rooster in each of 11 pens were infested with the chicken body louse, *Menacanthus stramineus* (Nitzsch). Eleven similar pens of birds were kept louse free as controls. No significant difference in egg production was observed between the two groups of hens during the 16 weeks following infestation. The louse-infested hens laid eggs at the 76.0% level while their louse-free counterparts produced at the 75.7% level. A reduction in the protein content of the feed from 16% to 12% was carried out for five "louse-infested" and five "louse-free" pens during the 17th week. The birds in the remaining 12 pens continued as before on the 16% protein feed. No significant difference was obtained when egg production data recorded during this 6-week period were analyzed as a split-plot design. The lice were removed at the end of the 22nd week of the test. Egg records for the ensuing 4 weeks showed no trend toward increased production over the birds that had been louse-infested. Fertility and hatchability were not adversely affected by the infestation of lice. Consecutive end-of-the-month louse counts of louse-infested hens located in an extra pen in the laying-hen test resulted in counts of 49, 1,575, 8,646 and 12,305, respectively.

Two adults of the chicken body louse were placed on each of 192 5-day-old broilers distributed evenly among four pens. An equal number of broiler chicks were located in adjacent pens. No significant difference in weight gain or in feed conversion was noted between the two groups of four pens at the end of the 8-week growth period. Birds were marketed at an average weight of 3 pounds.

Conflicting reports have appeared in the literature regarding the economic importance of the chicken body louse, *Menacanthus stramineus* (Nitzsch). In 1950 Edgar & King reported a 5.86% greater egg production by louse-free hens over an 11-month period. An infestation of *M. stramineus* lowered egg production by 15% over a 14-week period in tests reported by Gless & Raun (1959).

In contrast with the results of the above tests, Warren *et al.* (1948) reported that louse infestations did not in any way handicap the laying hen.

Experiments reported herein were designed to evaluate the actual damage caused by infestations of *M. stramineus*. Egg production records were analyzed to determine whether any reduction could be attributed to the louse infestation. Weight-gain and feed-conversion records were analyzed to determine the effect of an infestation of *M. stramineus* on a flock of broilers.

METHODS AND MATERIALS OF THE LAYING-HEN TEST.—An insulated poultry house measuring 12 by 39 feet was used in this experiment. Twenty-two pens were constructed within the building. Each pen was 2 feet wide, 6 feet long, 2 feet high and was raised 30 inches from the floor. Each was equipped with feeder and oyster-shell containers. A water trough fastened on the outside of the cage served two pens. One rollaway nest box, which projected out into the aisle, was furnished for each pen. Lights were timed to give a 13- to 14-hour day.

On October 9, 1958, four pullets and one rooster were placed in each pen. The birds were a result of a cross of two inbred lines of Leghorns and were furnished by the Ames-In-Cross Hatchery at Roland, Iowa. The chickens were wormed as they were randomly distributed among the 22 pens.

Eight pullets and two roosters, kept in a spare pen, were infested with lice. Three of these hens and one rooster were used to replace birds in the test when death occurred.

On November 16, 1958, the first egg was laid. Egg production records were kept for the ensuing 4 weeks. The pullets were then moved in order to pair pens of similar production.

On December 20, 1958, 3 *M. stramineus* were placed on each of the birds in 11 of the 22 pens. The pens of birds infested with lice were selected by the flip of a coin.

Egg production records were kept for 22 weeks after infestation. Daily egg production was recorded, and at the end of each 7-day period the egg numbers were totaled. The birds were fed a standard 16% laying mash purchased at a local elevator.

From the 17th through the 22nd week the protein content of the feed was reduced from 16% to 12% for the hens in five of the original 11 pairs of pens. The protein content was reduced gradually during a 10-day period to allow the birds to adjust to the changed feed.

At the end of the 22nd week the lice were removed. A 3-gallon compressed air sprayer was used to apply a 1% malathion emulsion on the birds. Application of this spray was made during semi-darkness to keep the birds from becoming overly excited. Egg records were kept for an additional 4-week period following removal of the lice, to determine whether any deviation in egg production could be noted in the birds formerly infested.

At intervals during the experiment the eggs laid over a 5- to 6-day period were saved to determine fertility and hatchability. Each egg was identified by pen number and stored in a walk-in-type cooler at 40° F. They were incubated at the Ames-In-Cross Hatchery at Roland, Iowa. Hatchery personnel culled the chicks at hatching time on the basis of salable qualities. The hatchery personnel did not know which eggs came from louse-infested hens.

Since louse-free and louse-infested birds were located in adjoining pens, frequent inspections were made of the birds that were to be kept louse-free. On two occasions one of these "louse-free" birds was found to have several lice. On those occasions, a 2% pyrethrum dust was applied by the pinch method to all birds that were to be kept louse-free.

RESULTS OF THE LAYING-HEN TEST.—For purposes of recording these data it was considered that an egg produced every day by each hen would be 100% production. Therefore, the percentage egg production would be the

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[2] Acknowledgment is made of the assistance given by the Ames-In-Cross Hatchery, Roland, Iowa, for the use of their hybrid broilers, hens, and hatching facilities.

**Table 1.—Egg production within treatments for the three periods of a laying-hen test. 1958–1959.**

| Weeks | Number of Pens | Treatment | Egg Production (%) |
|---|---|---|---|
| 1–16 | 11 | Louse-infested | 76.0 |
| | 11 | Louse-free | 75.7[a] |
| 17–22 | 5 | Louse-infested hens fed a 12% protein laying mash | 73.1 |
| | 5 | Louse-free hens fed a 12% protein laying mash | 69.3 |
| | 6 | Louse-infested hens fed a 16% protein laying mash | 73.4 |
| | 6 | Louse-free hens fed a 16% protein laying mash | 70.3[a] |
| 23–26 | 5 | Lice removed. Hens fed a 12% protein laying mash | 70.4 |
| | 5 | Louse-free hens fed a 12% protein laying mash | 56.4 |
| | 6 | Lice removed. Hens fed a 16% protein laying mash | 68.9 |
| | 6 | Louse-free hens fed a 16% protein laying mash | 70.2[a] |

[a] Contains an adjusted total to allow for 1 bird that laid no eggs during the entire experiment.

actual number of eggs laid, divided by the theoretically possible total. The louse-infested hens produced at the rate of 76.0% for the first 16 weeks of the experiment. The louse-free birds had an average 75.7% production for the same period (table 1).

The protein level in the feed had no apparent effect on the egg production of louse-infested or louse-free birds under the conditions of this test. Minor differences (table 1) were not significant when data obtained during this 6-week period were subjected to an analysis of variance using a split-plot design.

Removal of the lice had no apparent effect on egg production during the last 4 weeks of the test (table 1).

The fertility of both the louse-infested and louse-free birds was above 90% for all the eggs incubated during the entire test. A 3% difference in hatchability in favor of the louse-infested birds existed in the number of salable chicks resulting from all eggs set (table 2).

Chickens in the spare pen served another purpose. At 1-month intervals after initial infestation, the lice were removed from one of the birds in the spare pen and counted. A different bird was used for each end-of-the-month louse count. Pyrethrum dust was applied sparingly over the entire bird. The chicken was then placed in a cage, which had a wire bottom. A 1/4-inch mesh screen was placed 2 inches below the bottom of the cage to prevent feathers and manure from contaminating the lice, which were collected below on a large sheet of paper. Several times during the 4 hours required for removal of the lice the feathers were rubbed vigorously, causing nearly all the lice to fall to the paper. The larger foreign particles were removed after the lice had been poured into a beaker. The lice were then counted with the aid of a binocular wide-field microscope. The lice removed and counted from one bird at the termination of each month numbered 49, 1,575, 8,646 and 12,305 (fig. 1).

**Table 2.—Fertility and hatchability of eggs incubated in a laying-hen test. 1958–59.**

| Louse Status | Total Number of Eggs | | Fertility (%) | Salable Chicks From | | |
|---|---|---|---|---|---|---|
| | | | | Fertile Eggs | | All Eggs Set |
| | Set | Fertile | | Number | Per Cent | (%) |
| Infested | 1,013 | 933 | 92.1 | 830 | 89.0 | 81.9 |
| Free | 907 | 818 | 90.2 | 716 | 87.5 | 78.9 |

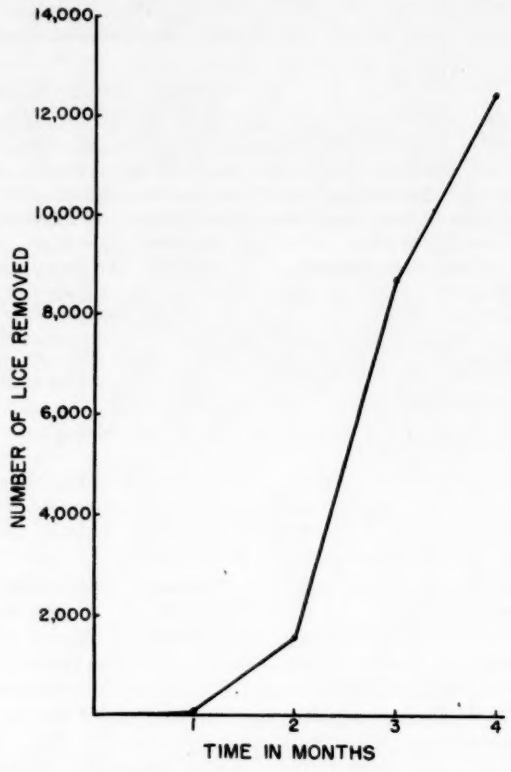


FIG. 1.—Number of lice removed from one bird 1 month, 2 months, 3 months, and 4 months after infestation.

DISCUSSION OF THE LAYING-HEN TEST.—At the end of the first 16 weeks of the test the egg production records indicated that the louse-infested birds were not affected adversely by the infestation. Therefore another factor was introduced into the tests by lowering the protein content of the feed. The change in protein content was made, since it could be more easily controlled than could a reduction in the amount of water or feed. The two factors together, (louse infestation and a 4% reduction in protein content of the feed) did not handicap the laying hens sufficiently to lower egg production. After the lice were removed, no definite trend in egg production took place.

A possible explanation of the opposite results reported by Gless & Raun (1959) in their egg production experiment is that genetic inbreds were used in their test. They found that the louse-infested hens produced 15% fewer eggs over a 14-week period. In contrast, the birds used in the experiment reported herein were the result of a cross of two inbred lines of Leghorns, and perhaps hybrid vigor was exemplified in a much higher rate of production. The approximate overall rate of production for the genetic inbreds used by Gless and Raun was 25% lower than the egg production rate reported in this paper, which is strong evidence that the genetic makeup of the birds in the two tests differed.

Louse counts made by Gless and Raun on two birds at the termination of their 14-week experiment revealed an average of 23,063 lice per bird. This number was significantly higher than the 8,646 and 12,305 lice that were re-

moved from two birds in this experiment after 12 and 16 weeks of infestation. The smaller louse population on these birds may be another explanation of why no apparent effect of the lice on egg production was found. Perhaps hybrid chickens can withstand the effects of a certain level of louse numbers, and the "threshold of effect" was not reached in this test.

It seems possible that poultry geneticists in their breeding program to develop a hen with higher producing ability, as well as a more rugged chicken which can withstand adverse environmental conditions, have also produced a bird that can withstand heavy infestation of *M. stramineus*. The differences in louse populations found on the inbreds used by Gless and Raun and the hybrid hens in this experiment indicate that hybrid chickens may not be as susceptible to a buildup of large numbers of *M. stramineus*.

METHODS AND MATERIALS OF THE BROILER TEST.—The poultry house used in this experiment measured 12 by 40 feet, with single walls and concrete floor. Eight pens, each measuring 4 feet wide, 9 feet long, and 3 feet high, were constructed within the building. Ground corncobs, to a depth of 4 to 6 inches, were used as litter. Each pen was equipped with a feeder, waterer, and a 150-watt heating lamp.

Placed in each pen on April 15, 1958, were 48 day-old chicks, 12 males and 12 females of each of two strains of broilers. The 384 chicks were supplied by the Ames-In-Cross Hatchery at Roland, Iowa. On April 19, 1958, the chicks in four of the pens were infested with lice. The chicks were 5 days old at this time. The selection of pens that contained birds to be infested with lice was accomplished by flipping a coin. Two adult *M. stramineus* were placed directly on each chick that was infested.

A high-energy broiler ration containing 22% protein was fed throughout the experiment. The feed was in a crumbled form during the first week and in a mash form during the remainder of the test.

Each week all birds were weighed individually. Total feed consumption per pen was recorded for the entire experiment.

A 2% pyrethrum dust was applied by the pinch method on two occasions when several lice were found on birds that were to be kept louse-free.

On June 14, 1958, at 8½ weeks of age, the birds had an average weight of 3 pounds. The lice were killed by spraying the entire house with a 1% malathion solution. The birds were then marketed.

RESULTS OF THE BROILER TEST.—The average weekly weights of the four pens of louse-infested birds and the four pens of louse-free birds are shown in figure 2. Louse-free birds averaged 0.34 ounce heavier than the louse-infested birds at the termination of the test. This difference was not considered significant.

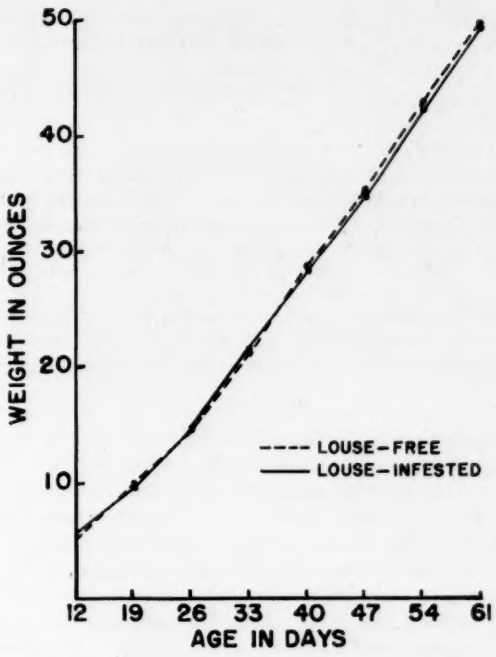


FIG. 2.—Average weekly weights of louse-infested and louse-free broilers.

Each of the louse-free broilers needed 2.238 pounds of feed to make 1 pound of gain, while louse-infested broilers consumed 2.232 pounds of feed per pound of gain. The 0.006 pound of feed difference is not significant.

DISCUSSION OF THE BROILER TEST.—The present-day method of confined rearing of broilers, especially bred for rapid growth, shortens the feeding period. In this experiment, *M. stramineus* evidently did not have time to build up sufficient numbers to be deleterious, since there were only approximately 1,500 lice per bird at the time of sale.

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# Some Characteristics of Alkali Bee Nesting Sites[1]

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Abstract

The alkali bee, *Nomia melanderi* Ckll., is an important pollinator of alfalfa in Wyoming. The number of nesting sites and the number of bees per nesting site have been declining in the Riverton seed-producing area during the past few years. An attempt has been made to characterize the sites chosen by bees so that promising but unproductive nesting areas may be modified in a manner to encourage bee utilization. Soil samples were taken and analyzed from several nesting and non-nesting areas near Riverton. Moisture in the sites was determined through the summer and early fall. In general, the selection of a particular site seemed to be at least partly dependent on suitable texture and proper moisture conditions. A soil of 75% sand, 17% silt and 8% clay, with a constant moisture of about 13%, seemed to be required. Other chemical and physical differences in the nesting and non-nesting areas were those associated with variations in the soil moisture. A scattering of plants encouraged nesting but was not absolutely necessary.

The alkali bee, *Nomia melanderi* Ckll., is a very efficient pollinator of alfalfa in Wyoming. It trips most flowers that it visits, while the honey bee, *Apis mellifera* L., trips one or two flowers in about 400 visits. When alkali bee populations are low, Wyoming alfalfa seed yields are low; in years of alkali bee abundance, the yields are comparatively high. Since 1955 alkali bee populations have steadily declined in the seed-growing area near Riverton, and seed yields have also declined. An attempt has been made to characterize the nesting sites chosen by bees so that promising but unproductive nesting areas may be modified in a manner to encourage bee utilization.

The distribution of the alkali bee in North America is given by Muesebeck *et al.* (1951) and the life history by Bohart & Cross (1955) and by Stephen (1959). Its activities as a pollinator have recently been reviewed by Bohart (1957). The alkali bee passes the winter as a prepupa inside its subterranean nest. In Wyoming it changes to the pupal stage in June and emerges as an adult in late June or early July. The female mates and constructs nests in the soil soon after its emergence. The bees remain active in the alfalfa fields and around the nesting sites until mid-August or a little later. The female is very selective in choosing a soil for nesting. Most nesting sites are in areas of water seepage and accumulation of soluble salts.

Methods.—To characterize such sites, soil samples were taken from the center of 11 nesting areas and from adjacent areas that lacked bee nests. These samples were analyzed at the Laramie Soils Laboratory. The following measurements were made as outlined in Richard (1954): (1) pH, saturated paste and diluted; (2) electrical conductivity of saturation extract; (3) cation-exchange-capacity; (4) exchangeable sodium; (5) exchangeable sodium percentage (E.S.P.); (6) soluble sodium; (7) soluble calcium and magnesium; (8) sodium adsorption ratio (S.A.R.); and (9) air-water permeability ratio (Ka ÷ Kw) at 24, 48, and 72 hours. In addition to these, measurements were made on (10) texture, by Bouyoucos hydrometer; (11) settling volume, by a modified Gardner (1945) method; (12) moisture equivalent by centrifuge method; and (13) per cent moisture by gravimetric method.

Results.—The results of these measurements are shown in table 1. The clay shown in this table is made up of particles less than 0.002 mm., silt 0.002 to 0.02 mm., and sand 0.02 to 2 mm.

According to Stephen (1959), good bee-nesting sites never contain more than 8% clay in the silty Oregon soils.

**Table 1.—Physical and chemical soil characteristics from alkali bee nesting sites and adjacent areas where bees were absent. Riverton, Wyoming, 1958–1959.**

| Characteristic | Bees Present | | Bees Absent | |
|---|---|---|---|---|
| | Average | Range | Average | Range |
| Sand % 0.02–2.00 mm. | 74.6 | 84–68 | 74.0 | 86–63 |
| Silt % 0.002–0.02 mm. | 16.8 | 21–10 | 15.5 | 26–8 |
| Clay % <0.002 mm. | 8.6 | 18–2 | 10.5 | 16–2 |
| pH saturated | 7.71 | 8.0–6.9 | 7.95 | 8.2–7.2 |
| $pH_s$ diluted | 8.10 | 8.6–7.2 | 8.47 | 9.0–7.5 |
| $EC_e$, E.C. $\times 10^3$ | 14.96 | 30.00+–0.70 | 9.00 | 30.00+–0.40 |
| C. E. C. Meq./100 gm. | 8.06 | 10.96–6.32 | 8.05 | 9.44–6.20 |
| Exchangeable Na Meq./ 100 gm. | 1.28 | 2.71–0.99 | 1.72 | 7.05–0.41 |
| Exchangeable Na (%) | 15.76 | 27.90–7.26 | 20.54 | 73.79–4.97 |
| Soluble cations | | | | |
| Na Meq./100 gm. | 2.32 | 4.823–0.072 | 0.896 | 3.931–0.026 |
| Ca+Mg. Meq./100/gm. | 2.78 | 11.228–0.216 | 1.10 | 2.127–0.128 |
| S. A. R. | 2.39 | 5.78–0.22 | 1.22 | 4.57–0.10 |
| Settling volume ml. | 16.0 | 18–15 | 16.0 | 17–14 |
| Moisture equivalent (%) | 9.80 | 10.07–7.42 | 9.87 | 11.19–7.39 |
| Ka ÷ Kw | | | | |
| 24 hours | 6.92 | 10.57–3.68 | 6.51 | 9.77–4.23 |
| 48 hours | 6.24 | 8.18–4.03 | 6.74 | 10.38–3.86 |
| 72 hours | 6.59 | 10.02–4.55 | 6.85 | 11.45–4.07 |
| Moisture (%) | 12.48 | 14.81–9.45 | | |
| Dry areas | | | 8.75 | 10.24–7.56 |
| Wet areas | | | 16.08 | 17.06–15.21 |

This percentage was low for Wyoming conditions, where the sandy nesting sites ranged between 2% and 18% clay. However, the poor sites did contain a higher percentage of clay than the good sites.

The salinity, as measured by electrical conductivity, and the soluble sodium were higher in the nesting sites than in the non-nesting areas. The exchangeable sodium percentage was higher in the non-nesting sites. The soluble salts are known to increase the water-holding capacity of the soil and to influence the vegetation found on the soil. These salts may also cause crusting of the surface soil layer and thus interfere with evaporation and movement of soil water.

In addition to the moisture determinations made at the time these samples were taken, moisture samples were taken through July, August, and part of September from four areas having bees and from the immediately surrounding area without bees. In one of the bee sites, the bees started nesting but later abandoned the area. Five samples were taken from each area by means of a soil auger driven 8 inches into the soil. The soil was placed

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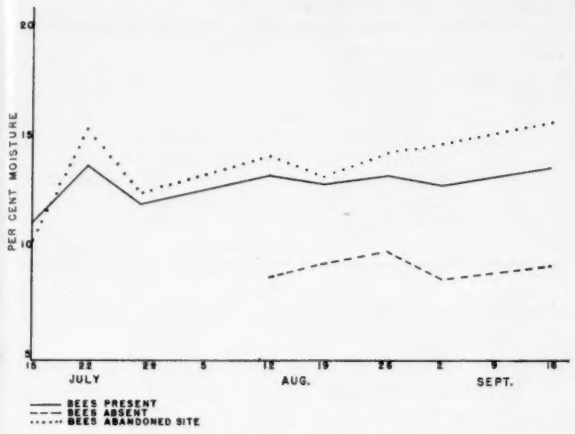


FIG. 1.—Soil moisture in alkali bee nesting sites and comparable areas where bees were absent. Riverton, Wyoming, 1958–59.

in a tight can, weighed, dried in an oven, and weighed again to determine the loss of water by drying. The results are shown in figure 1.

The areas having active bee sites had a moisture content ranging between 12% and 13.5%. Non-nesting areas were much drier, having moisture between 8% and 9.75%. In one site, the bees started nesting when the moisture was 11%. This site gradually became more moist, and bee activity ceased when the moisture exceeded 14%. The site dried to between 13% and 14% but the bees did not return. Stephen (1959) found the best sites to contain 20% to 25% moisture. In the sandy soils of Wyoming, however, bees were never observed nesting in areas that had much above 14% moisture.

The best nesting sites were in areas having at least 27 inches of soil above bed rock, but some sites were found in areas having only 18 inches of soil.

The number of nests in the nesting sites ranged from 2 to 48 per square foot with an average of 19.5. Bees usually selected sites with scattered vegetation. The pioneer bee usually nested close to a plant and other bees would then nest close to the first nest. Where there were 7 to 13 small slumps of grama grass (*Bouteloua gracilis* H.B.K. Lag.) per square foot, the number of bee nests per square foot was 23 to 30. No bees were found where there were more than 13 clumps of grass per square foot. One site heavily covered with kochia (*Kochia scoparia* L.) had an average of three nests per square foot. Where there were more than 62 young kochia plants, no bees were found. In areas bare of vegetation there was an average of 18 nests per square foot.

CONCLUSIONS.—From this study it is indicated that the chief factor in nesting-site selection in the sandy soils of Riverton centers around soil moisture and the factors associated with it. The texture needs to be of a nature that can be worked by the bee and must also have certain water-holding properties. Desirable soils for nesting normally contain soluble salts, but the salts seem to be of little importance as far as site selection is concerned. It would appear that, under the conditions found at Riverton, Wyoming, the best site for bee nesting would be an area having approximately 75% sand, 8% clay and 17% silt, and a moisture content of 13%. A scattering of plants encourages nesting but is not absolutely necessary.

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# Acute Toxic Effects upon Livestock and Meat and Milk Residues of Dieldrin[1]

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ABSTRACT

Dieldrin was found to produce poisoning when applied dermally to young calves at 0.25%, young lambs at 3%, cattle at 2% and sheep and goats at 4%; lower concentrations did not produce poisoning under the conditions of this study. Higher concentrations were toxic in all cases. Horses tolerated 1% sprays.

When orally administered, dieldrin produced poisoning of young calves at 10 mg./kg., young pigs at 50 mg./kg., sheep at 25 mg./kg. and horses at 25 mg./kg.; lower doses were not toxic.

All treatments, single and multiple, produced residues in meat or milk. Fat analysis showed levels of 8 to 146 parts per million (p.p.m.) following treatment; milk showed 4.9 to 8.3 p.p.m. following 0.5% sprays.

Residues declined slowly, 16 to 24 weeks being required for fat to reach less than 1 p.p.m., 3 weeks for milk to return to less than 0.2 p.p.m.

A reasonably general survey of the toxicity and meat and milk residues of dieldrin for domestic animals has been completed during 1949 and 1950, at Kerrville, Texas, by the Entomology Research Division and the Animal Disease and Parasite Research Division of the U. S. De-

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[2] Animal Disease and Parasite Research Division.
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Table 1.—Results of spray treatments of baby Jersey calves with dieldrin.

| No. Treated | Formulation | Dieldrin Concentration (%) | Onset of Symptoms (hrs.) | Treatment to Death Interval | Final Result |
|---|---|---|---|---|---|
| 8 | Emulsion | 1.0 | 8– 50 | 11–144 hrs. | All died |
| 4 | Emulsion | 0.75 | 20– 80 | 1–9 days | 3 dead, 1 unaffected |
| 4 | Emulsion | 0.5 | 96–166 | 7–9 days | 2 dead, 2 unaffected |
| 5 | Wettable powder | 0.5 | 336 | 340 hrs. | 3 affected, 2 died, 2 unaffected |
| 3 | Wettable powder | 0.5 | 72 | 76 hrs. | 1 dead, 2 unaffected |
| 8 | Emulsion | 0.25 | 24–264 | 48–270 hrs. | 5 dead, 3 unaffected |
| 8 | Emulsion | 0.1 | — | — | 8 unaffected |

partment of Agriculture in large measure in formal cooperative agreement with Julius Hyman & Company (later absorbed by Shell Chemical Corporation). The results of these studies are described in this paper.

*Experimental Animals.*—The cattle, sheep, goats, hogs, and horses used in these experiments were purchased from owners able to certify that no previous insecticide treatment had been given them. All animals were examined carefully to assure that only those free of disease would be used. Considerable difficulty was experienced in finding baby calves free of enteric disorders, but it is believed that this condition was held to minimal effect in these experiments. All animals were well fed and adequately housed.

*Experimental Procedure.*—External applications of dieldrin were made either by spraying or dipping. For dipping, a small tank was used and the animals were submerged as in ranch practice. Spraying called for saturating the animal's hair and skin surface with the particular formulation at 60 to 180 pounds per square inch pressure. A considerably greater quantity than that retained on the animal was applied and allowed to run off to insure complete wetting of hair and skin.

Oral doses of dieldrin were administered by means of drenching syringes or by stomach tube.

*Experimental Materials.*—Two basic formulations of dieldrin were used in these studies, an emulsifiable concentrate and a wettable powder. The concentrate for emulsions was composed of 25% dieldrin, 65% xylene, and 10% Triton X-100 by weight. For the various emulsions, this concentrate was diluted with ordinary well water on a weight/weight basis.

The wettable powder used was composed of dieldrin, 25%; Attapulgus clay, 74.5%; and Atlox 1045, 0.5%, by weight. Suspensions of this powder were prepared in well water on a weight/weight basis.

*Analyses.*—Under terms of the formal agreement between the U. S. Department of Agriculture and Julius Hyman & Company, chemists of the Hyman Company handled the larger proportion of the analyses and were checked at unstated intervals by chemists of the Entomology Research Division. The results of analyses reported in this paper are those furnished by chemists of the Julius Hyman organization.

*Observations for Toxic Effects.*—Close clinical observations were made of all treated animals. For purposes of this study an animal was considered as affected whether it demonstrated severe convulsions or minor muscular spasms, since either manifestation is a toxic reaction.

If death occurred, an autopsy was performed as soon thereafter as possible, usually within the hour. Gross lesions were recorded and tissues collected for micropathological study.

Phase *I. Acute Toxicity of Dieldrin for Baby Calves.*—

A. Externally applied: The calves used in this study were all from 1 to 2 weeks old and were Jersey or Jersey crossbreeds. All were maintained on a diet of diluted evaporated milk fed from nipple buckets. Applications of dieldrin were usually made within 2 to 4 hours after feeding. Results of the various treatments are summarized in table 1. It may be noted that there was a wide variation in susceptibility of individual animals and a similar variation in the time required for initial symptoms to appear and for the animal to succumb after initial symptoms appeared.

B. Internally administered by dosing syringe: These calves were penmates of those used in A, Phase I and were similarly maintained. Administration was usually 2 to 4 hours after feeding. Results are summarized in table 2. In these cases, it may be noted that very little variation occurred between times of onset of symptoms or of death.

Phase *II. Acute Toxicity of Externally Applied Dieldrin for Baby Lambs.*—The lambs used in these tests were nurslings maintained by the mother ewe. All were approximately 2 weeks old. Results of the applications are summarized in table 3. It may be noted that wide variations in the treatment-to-onset of symptoms and treatment-to-death intervals occurred as they did in the calf experiments.

Phase *III. Acute Toxicity of Dieldrin for Pigs.*—

A. Externally applied: Four weaned Poland-China pigs 8 weeks old were sprayed to saturation with 4% dieldrin emulsion (approximately 1,200 mg./kg.). None were affected.

Table 2.—Results of oral dosages of dieldrin administered as a 5.0% emulsion.

| Calf Number | Dose (mg./kg.) | Weight (kg.) | Onset of Symptoms (hrs.) | Treatment to Interval (hrs.) | Final Result |
|---|---|---|---|---|---|
| 1 | 25 | 32.2 | 5 | 7 | Dead |
| 2 | 15 | 24.7 | 3 | 8 | Dead |
| 3 | 15 | 26.5 | 5 | 10 | Dead |
| 4 | 10 | 22.7 | 5 | 5.5 | Dead |
| 5 | 10 | 28.2 | — | — | Unaffected |
| 6 | 10 | 25.4 | 5 | 7 | Dead |
| 7 | 5 | 26.7 | — | — | Unaffected |
| 8 | 5 | 26.5 | — | — | Unaffected |

**Table 3.—Results of external application of dieldrin to animals.**

| No. Treated | Formulation[a] | Dieldrin Concentration (%) | Treatment to: Symptoms Interval | Treatment to: Death Interval | Final Result |
|---|---|---|---|---|---|
| | | | *2-week-old Delaine lambs* | | |
| 3 | E spray | 3.0 | 5–96 hrs. | 72–144 hrs. | 3 dead |
| 5 | WP dip | 3.0 | 3 hrs.–14 days | 4 hrs.–19 days | 4 dead, 1 affected and recovered |
| 2 | E spray | 2.0 | — | — | Unaffected (3-day observation) |
| | | | *Angora sheep and goats* | | |
| 2 | Sheep-WP dip | 4.0 | 22–48 hrs. | — | Both affected[b] |
| 2 | Sheep-E spray | 4.0 | 4–5½ hrs. | — | Both affected[b] |
| 4 | Goats-E spray | 4.0 | 28 hrs. | 8 days | 1 dead, 3 unaffected |
| | | | *Yearling Jersey steers* | | |
| 2 | E spray | 4.0 | 5–7½ hrs. | — | 2 affected[b] |
| 2 | E spray | 3.0 | 5–24 hrs. | — | 2 affected[b] |
| 2 | E spray | 2.0 | 3½–5 hrs. | 61 hrs. | 2 dead |
| 2 | WP spray | 2.0 | 23–26 hrs. | — | 2 affected[b] |
| 2 | E spray | 1.0 | — | — | Unaffected |

[a] E, emulsion; WP, wettable powder.
[b] Both recovered.

B. Internally administered by dosing syringe: A Hampshire sow nursing 10 pigs 10 to 14 days old was obtained for this work. A 5% (w/v) emulsion was used to measure the desired dosage. The required quantity was diluted equally with evaporated milk before administration. Results are given in table 4. Some brief nausea resulted from the xylene in the formulation.

PHASE *IV. Acute Toxicity of Externally Applied Dieldrin for Jersey Steers.*—

One-year-old Jersey steers were used for this work. They were kept in pens and fed alfalfa hay. Results of applications are summarized in table 3. It may be noted that wide variation of individual susceptibility appeared to exist in cattle although little variation occurred in the treatment to onset-of-symptoms interval.

PHASE *V. Acute Toxicity of Dieldrin for Sheep.*—

A. Externally applied: For this study 1-year-old Delaine muttons were used, fed a diet of alfalfa hay. Results are summarized in table 3.

B. Internally administered: These sheep were similar to those used in A, Phase V, and were maintained on the same diet.

1. One yearling mutton weighing 28.5 kg. was given 25 mg./kg. of dieldrin in the form of a 1% emulsion. This sheep became affected 3 hours after administration but showed only mild symptoms until the following day, at which time convulsions began and persisted intermittently for 2 days. After convulsions had subsided, the sheep appeared stunned and blinded for 24 hours, then recovered without event.

2. One yearling mutton weighing 54.7 kg. was given 15 mg./kg. of dieldrin as a 1% emulsion. This sheep was unaffected during 8 days of observation.

**Table 4.—Results of orally administered doses of dieldrin on 14-day-old-pigs (Hampshire).**

| Pig No. | Dose (mg./kg.) | Weight (kg.) | Treatment to: Symptom Interval (hrs.) | Treatment to: Death Interval | Final Results |
|---|---|---|---|---|---|
| 1 | 5 | 6.59 | — | — | Unaffected |
| 2 | 5 | 6.14 | — | — | Unaffected |
| 3 | 10 | 4.54 | — | — | Unaffected |
| 4 | 10 | 5.91 | — | — | Unaffected |
| 5 | 25 | 7.27 | — | — | Unaffected |
| 6 | 25 | 6.7 | — | — | Unaffected |
| 7 | 50 | 6.02 | 2 | — | Affected and recovered |
| 8 | 50 | 6.81 | 2 | 4 days | Dead |

PHASE *VI. Acute Toxicity of Dieldrin Externally Applied to Angora Goats.*—

The four Angora does used in this experiment were 2 years old and in full fleece. One of the goats, unaffected by dieldrin, died of exposure in a sudden cold rain. Results are summarized in table 3.

PHASE *VII. Acute Toxicity of Dieldrin for Horses.*—

A. Externally applied: Three horses, 4, 8, and 15 years old (cow pony type) were sprayed to saturation with a 1% emulsion of dieldrin (approximately 66 mg./kg.). Neither of these horses became affected.

B. Internally administered: Dieldrin in suspension as a wettable powder was administered by means of a stomach tube to one horse 8 years old, weighing 400 kg. The dosage of dieldrin was 25 mg./kg. This horse showed typical muscular spasms and twitches, together with staggering and dizziness 3 hours after administration, but recovered within 12 hours.

PHASE *VIII. Residues of Dieldrin in the Fat of Hereford Steers Following Spraying with Dieldrin.*—

A. Residues following 0.5% sprays: Four Hereford steers approximately 8 months old were purchased and fed on a fattening ration. One pair was sprayed with 0.5% dieldrin emulsion and the other pair was sprayed with dieldrin suspension containing 0.5% of the active ingredient. This dosage was approximately 33 mg./kg. of dieldrin per treatment. Three applications were made, the second 2 weeks after the first, and the third 3 weeks after the second. One of the steers sprayed with the suspension of dieldrin suffered mild nervous attacks 17 days after the second application and very typical convulsions 3 days after the third application. One emulsion-sprayed steer

**Table 5.—Residues of dieldrin in omental fat of Hereford cattle sprayed three times in 5 weeks with 0.5 % dieldrin.**

| Animal No. | Dieldrin in Omental Fat (p.p.m.) (weeks after third spray) | | | |
|---|---|---|---|---|
| | 6 | 15 | 19 | 24 |
| 1 | 70 | 11 | 10 | 0.0–1 |
| 2 | 58 | 6 | 6 | .0–1 |
| 3 | 38 | 6 | 4 | .0–1 |
| 4 | 8 | 4 | 8 | .0–1 |

showed mild disturbance on the day following the third application. All recovered without other evidence of injury.

An omentectomy was performed on each of these four steers prior to application of any dieldrin. The omental fat so removed served as a control in subsequent analytical procedure. From first spraying until final biopsy (240 days), these steers averaged 316 pounds gain. The steer most severely affected by the sprays gained 300 pounds. The steers were subjected to omentectomies 6, 15, 18, and 24 weeks after the third spraying. Results of analyses of the fat samples, determined by infra-red spectroscopy, are given in table 5.

B. Residues following 0.25% sprays: Six Hereford steers and two Hereford heifers 8 months old were purchased and fed a fattening ration. Three steers and one heifer were sprayed with 0.25% emulsion of dieldrin eight times at 3-week intervals. The remaining three steers and one heifer were sprayed eight times at 3-week intervals with 0.25% dieldrin suspension. These sprays represented dieldrin dosages of approximately 15 mg./kg. per treatment. No toxic reactions occurred in any of the animals.

One-half (four) of the cattle were subjected to omentectomy before spraying began, the omental fat so obtained being held as control samples for later analytical work. Three weeks after each spraying, two animals from each group of four were again subjected to omentectomy, alternating pairs within a group. Additional omentectomies were performed 6, 9, 12, 15, and 16 weeks after the final spraying.

From November 20, 1949 to April 26, 1950, covering the period of spray applications, the cattle averaged 283.8 pounds of gain in spite of a total of 36 omentectomies performed upon the eight animals. Residues in the omental fat at various intervals, as determined by infra-red spectroscopy, are given in table 6.

PHASE *IX. Contamination of Milk by Spraying Dairy Cows with Dieldrin.*—

1. One Jersey cow was sprayed twice at a 3-week interval with a 0.5% emulsion of dieldrin. A second cow was sprayed with a 0.5% suspension of dieldrin at the same intervals. These applications approximated a dieldrin dosage of 33 mg./kg. Milk samples were obtained in duplicate from each cow on the day before the first spraying and 1, 2, 3, 7, 14, and 21 days after each spraying. Each sample consisted of 250 gm. of whole milk with 1 cc. of 37% formalin added as a preservative. Analyses of these samples were failures and the experiment was repeated. No toxic reactions in the cows were observed.

2. One Jersey cow was sprayed with 0.5% dieldrin emulsion and a second cow with 0.5% dieldrin suspension (approximately 33 mg. dieldrin per kilogram). Milk samples, each 200 gms. and in duplicate, were taken 6 and 3 days before treatment, and 1, 2, 3, 4, 7, 14, 21, 28, 35, and 42 days after treatment. These samples were preserved with 0.4 cc. of 37% formaldehyde. Duplicate samples of untreated milk were prepared for controls. The milk samples provided from this experiment were assayed by biological means, using house flies (*Musca domestica* L.) as the test insects. Dieldrin content of the samples as reported by Y. P. and Jean Sun, Biological Laboratory, and Julius Hyman & Company are given in table 7.

**Table 6.—Residues of dieldrin in omental fat of Hereford cattle sprayed at 3-week intervals with 0.25% dieldrin.**

| Animal No. | Dieldrin in Omental Fat (p.p.m.) (3 weeks after number of sprays) | | | | | | | (weeks after eighth spray) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 6 | 9 | 12 | 15 | 16 |
| 61 | | 16 | | 12 | | 24 | | 13 | | 1 | | 0.0–1 |
| 62 | | 22 | | 8 | | 37 | | 8 | | 5 | | .0–1 |
| 63 | 23 | | 14 | | I[a] | | 19 | | 18 | | | |
| 64 | I[a] | | 10 | | Lost | | 11 | | 9 | | 1.6 | |
| 65 | | 15 | | 5 | | 22 | | 12 | | 10 | | .0–1 |
| 66 | | 4 | | 30 | | 35 | | 14 | | I | | 3.8 |
| 67 | 10 | | 13 | | 13 | | 14 | | 10 | | 1.2 | |
| 68 | I[a] | | 11 | | 10 | | 13 | | | | | |

[a] Interference, no accurate infrared reading possible.

PHASE *X. Miscellaneous Dieldrin Residue Studies.*—Without specific planning, during the studies of the acute toxicity of dieldrin, certain samples of fat were taken for analysis. Results of infra-red spectroscopic analyses performed by Julius Hyman & Company are summarized in tables 8 and 9. These samples represent animals killed by dieldrin, animals intoxicated without death, and animals showing no ill effects of the treatment applied. It may be observed in studying tables 3 and 4 that the quantity, in terms of parts per million of dieldrin, found in fat cannot be correlated directly with the intensity of effect upon the

**Table 7.—Dieldrin present in milk of dairy cows after spraying with 0.5% dieldrin.**

| Cow No. | Formulation | Sample (Days after Spraying) | Dieldrin Found (p.p.m.)[a] |
|---|---|---|---|
| 5 | Xylene emulsion | 1 | 5.7 |
| | | 2 | 8.3 |
| | | 3 | 7.6 |
| | | 4 | 5.5 |
| | | 7 | 5.3 |
| | | 14 | 2.7 |
| | | 21 | 1.5 |
| | | 28 | 1.0 |
| | | 35 | .18 |
| 6 | Wettable powder susp. | 1 | 2.2 |
| | | 2 | 4.2 |
| | | 3 | 2.9 |
| | | 4 | 3.2 |
| | | 7 | 2.2 |
| | | 14 | 1.5 |
| | | 21 | .8 |
| | | 28 | .4 |
| | | 35 | .05 |

[a] As determined by biological assay.

**Table 8.—Dieldrin residues in tissues from Jersey steers and calves given lethal doses.[a] 1949.**

| Source of Sample | Dieldrin Emulsion (%) | Date of Application | Date of Death | Dieldrin (p.p.m.) |
|---|---|---|---|---|
| Omental fat, steer No. 1 in Phase IV-1 | 2 | Aug. 9 | Aug. 12 | 75 |
| Omental fat, steer No. 2 in Phase IV-1 | 2 | Aug. 9 | Aug. 12 | 146 |
| Omental fat, calf No. 2 in Phase I-A-1 | 1 | Aug. 3 | Aug. 4 | 54 |
| Omental fat, calf No. 3 in Phase I-A-1 | 1 | Aug. 3 | Aug. 4 | 38 |
| Kidney fat, calf No. 1 in Phase I-A-1 | 1 | Sept. 3 | Sept. 14 | 55 |
| Hair, calf No. 1 in Phase I-A-1 | 1 | Sept. 3 | Sept. 14 | 0.54[b] |

[a] Samples taken at autopsy.
[b] Per cent by weight.

**Table 9.—Dieldrin residues in omental fat of Jersey steers determined from biopsy samples. 1949.**

| Identity Steer | Dieldrin Treatment | Date of Application | Date of Biopsy | Dieldrin (p.p.m.) |
|---|---|---|---|---|
| No. 1, Phase IV-4 | 2% suspension | Sept. 13 | Oct. 7 | 12 |
| No. 2, Phase IV-4 | 2% suspension | Sept. 13 | Oct. 7 | 93 |
| No. 1, Phase IV-2 | 3% emulsion | Aug. 8 | Aug. 23 | 75 |
| No. 1, Phase IV-5 | 1% emulsion | Aug. 10 | Aug. 23 | 119 |
| No. 2, Phase IV-5 | 1% emulsion | Aug. 10 | Aug. 23 | 67 |

animal. These results are presented only as additional data.

*Symptoms of Dieldrin Poisoning.*—In general, the symptoms of dieldrin poisoning are similar to those produced by chlordane and toxaphene. Premonitory evidences are hyperexcitability, nervousness, and mild salivation, followed soon by spasms of various muscle groups, those of the neck and face usually being first affected. The spasms generally increase in intensity and frequency until convulsions result. The animal may fall or stand during such seizures. Chewing movements with profuse salivation are seen. Crying out during convulsions is common. Death may occur following such attacks.

The incubation period of dieldrin poisoning may vary from a few hours to several days. In addition, fatal and nonfatal seizures may recur several days after apparent recovery.

As with other insecticides of the chlorinated hydrocarbon series, wide species variations in susceptibility exist. In cattle, increased resistance occurs with age, but in sheep little difference is apparent in the doses toxic to young and old.

*Autopsy Lesions of Dieldrin Poisoning.*—Body temperature at time of death may reach 114° F. if the animal dies in convulsions. The skin is usually denuded over the bony prominences. The visible mucous membranes are usually cyanotic and an accumulation of saliva is present under the head and on the lips. The abdominal viscera generally show a whitish discoloration owing to the high body temperature. The liver and kidneys are generally congested. Occasionally, hemorrhages occur at the junction of the abomasum with the remainder of the stomach. Hemorrhages along the intestines are not common. Almost always extensive hemorrhage appears beneath the epicardium. Occasionally, hemorrhages occur beneath the endocardium. Generally, the lungs are heavily congested and edematous.

*Micropathology.*—The most prominent effect of dieldrin, aside from generalized cloudy swelling in most organs, is displayed in the kidney tubules. Vacuolation and various degenerations appear rapidly in the tubules. Proliferation of capsular epithelium with desquamation is frequently observed. Liver cells show vacuolation and subsequent necrosis, but usually only after a longer period than the kidneys. Other microscopic findings are those common to prolonged convulsions and high body temperature.

# Toxicity to House Fly Larvae of Droppings from Chickens Fed Insecticide-Treated Rations[1]

MARTIN SHERMAN and ERNEST ROSS[2]

ABSTRACT

The toxicity to house fly (*Musca domestica* L.) larvae of feces from chickens on a continuous dietary regimen of laying ration containing Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), malathion, phenothiazine, ronnel, and Dow ET-15 (*O*-methyl *O*-(2,4,5-trichlorophenyl) phosphoramidothioate) was investigated. The insecticide levels in the feed that caused greater than 90% mortality in larvae placed in the manure were: Co-Ral, 89 p.p.m.; Diazinon, 154 p.p.m.; Dipterex, 89–132 p.p.m.; ronnel, 176–220 p.p.m.; and Dow ET-15, 89 p.p.m. Feces from chickens on malathion- and phenothiazine-treated rations caused relatively low mortality in the larvae at concentrations as great as 1,102 p.p.m. and 11,023 p.p.m., respectively.

The breeding of house fly (*Musca domestica* L.) larvae in poultry droppings is a serious problem. The application of larvicides by conventional methods is generally

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effective for a comparatively short period. However, the use of the chicken as a vehicle for the distribution of insecticide in droppings could offset many of the difficulties inherent in the conventional methods of application. The chicken voids both urine and feces through a common opening, the cloaca, resulting in a more or less intimate mixture of these excretory products. Thus, most of the insecticide and its metabolites are subsequently found well distributed throughout the droppings and are available for larvicidal activity.

In a study with chicks, Sherman and Ross (1959) administered single oral doses of six insecticides to 1-week-old New Hampshire cockerels and studied the toxic effects of the droppings from these chicks on house fly larvae. Their data showed that levels of insecticide that were nontoxic to the chicks caused high larval mortality for periods ranging from 1 to 6 days after administration.

This 29-week study was undertaken to investigate the effect on house fly larvae of the droppings from chickens fed a continuous dietary regimen of insecticide-treated laying mash.

MATERIALS AND METHODS.—Four separate feed mixes were made during the course of this study. The initial insecticide-feed mixtures were made June 24, 1958. The feed used in this study was a standard layer ration. The composition and concentrations of the insecticides included in the mixes at this time were as follows:

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), technical, 97%, 22, and 11 p.p.m.; Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate), technical, 89% with 11% related compounds, 22 and 11 p.p.m.; Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), technical, 100%, 89 and 44 p.p.m.; malathion, technical, 95%, 220 p.p.m.; phenothiazine, wettable powder containing 98.5% of the active ingredient, 6,613 p.p.m.; ronnel, wettable powder containing 25% of the active ingredient, 89 and 22 p.p.m.; Dow ET-15 (*O*-methyl *O*-(2,4,5,-trichlorophenyl)phosphoramidothioate), technical, 22 and 11 p.p.m.

Fifty pounds of insecticide-treated ration were prepared. Each insecticide was dissolved or suspended in acetone and then sprayed onto 1 pound of feed in a small blender. The mixed feed was spread on paper and the acetone allowed to evaporate. The resulting premix was then hand mixed with a larger amount of feed and further mixed for 15 minutes in a vertical type feed mixer with the balance of the ration.

On July 22, 1958, another 50 pounds of each treatment ration was prepared. At this time the concentrations of most of the insecticides were increased to obtain a greater toxic action to house fly larvae in the manure from the treated birds. The formulations of the insecticides were kept constant, however. The toxicants and subsequent changes in concentrations were as follows:

Co-Ral, 22 p.p.m. increased to 89 p.p.m., and 11 p.p.m. increased to 44 p.p.m.; Diazinon, 22 p.p.m. increased to 89 p.p.m., and 11 p.p.m. increased to 44 p.p.m.; Dipterex, 89 p.p.m. maintained at the same level but 44 p.p.m. increased to 132 p.p.m.; malathion, 220 p.p.m. increased to 440 p.p.m.; phenothiazine, 6,613 p.p.m. increased to 11,023 p.p.m.; ronnel, 89 p.p.m. maintained at the same level but 22 p.p.m. increased to 132 p.p.m.; and Dow ET-15, 22 p.p.m. increased to 132 p.p.m., and 11 p.p.m. increased to 89 p.p.m.

On August 14, 1958, treatment rations were prepared in 100-pound lots. At this time two of the formulations as well as the test concentrations were changed. Co-Ral and Diazinon were changed from the technical materials to wettable powder formulations containing 25% of the active ingredients. The concentrations of insecticides prepared at this time were as follows:

Co-Ral, 89 p.p.m. maintained at the same level but 44 p.p.m. increased to 132 p.p.m.; Diazinon, 89 p.p.m. increased to 220 p.p.m., and 44 p.p.m. increased to 154 p.p.m.; Dipterex maintained at 89 p.p.m. and 132 p.p.m.; malathion, 440 p.p.m. increased to 1,102 p.p.m.; phenothiazine maintained at 11,023 p.p.m.; ronnel, 89 p.p.m. increased to 220 p.p.m., and 132 p.p.m. increased to 176 p.p.m.; and Dow ET-15 maintained at 89 and 132 p.p.m.

The final treated-ration mixtures were made on September 11, 1958, when 300-pound lots were prepared. The formulations and concentrations were the same as those prepared on August 14. The animals were maintained on these feed mixtures until January 13, 1959, at which time the experiment was terminated.

The New Hampshire breed of chickens used in this study were kept in two separate lots. When the study was initiated one lot was composed of forty-eight 9-day-old chicks of mixed sex and the other was composed of fifty-two 48-week-old hens. The hens were assigned at random to individual laying cages. The chicks were kept in individual cage brooders until they were 8 weeks old and were then transferred to individual laying cages. Each experimental treatment was assigned at random to four birds in each lot. Since there were fewer chick cages than hen cages available, one Dipterex treatment was eliminated. Water and treated-feed were supplied *ad libitum*.

Feces[3] from all birds were assayed for toxicity to house fly larvae. In sampling the droppings, no collection was taken earlier than 3 days after a change was made in the insecticide concentration of the ration. Metal trays were placed beneath each cage to collect the droppings from the birds. Since the cages were in unscreened buildings, the feces deposited in the trays were available to volunteer muscoid flies for oviposition. To keep such oviposition at a minimum, the evening before a larval toxicity test was to be made the trays were removed, carefully scraped, and cleaned of droppings before being replaced beneath the cages. On the following morning, the droppings from each bird were scraped separately from the pans into half-pint waxed cartons and taken into the laboratory. A fecal sample of 15 to 20 gms. was taken from each carton, placed in a Stender dish, and approximately 2 ml. of distilled water added. Ten newly hatched house fly larvae were placed in each fecal sample. The dish was then covered with a fine muslin cloth to prevent the escape of the larvae and stored 3 days at a temperature of 25° C. Two days after the introduction of the larvae, an additional 2 ml. of water were added to each dish to replace moisture lost by evaporation. Three days after the introduction of the larvae into the fecal sample the number of survivors was determined.

[3] Inasmuch as the feces and urine are voided through a common vent, the terms feces, droppings, or manure, as used in this paper, refer to the mixture of the two.

**Table 1.—Toxicity to house fly larvae of manure from hens and chicks on insecticide-treated rations.**

| Insecticide | Concentration[a] in Feed Mixture (p.p.m.) | Hen | | Chick | |
|---|---|---|---|---|---|
| | | No. Larvae Tested | Corrected Mean Per Cent Mortality | No. Larvae Tested | Corrected Mean Per Cent Mortality |
| Co-Ral | 132 | 120 | 97.2 | 40 | 100 |
| | 89 | 240 | 93.2 | 150 | 96.0 |
| | 44 | 120 | 82.6 | 120 | 68.7 |
| | 22 | 120 | 47.5 | 40 | 10.5 |
| | 11 | 120 | 12.9 | 40 | 23.7 |
| Diazinon | 220 | 120 | 100 | 40 | 100 |
| | 154 | 120 | 100 | 40 | 100 |
| | 89 | 120 | 59.1 | 120 | 57.2 |
| | 44 | 120 | 19.9 | 120 | 11.7 |
| | 22 | 120 | 42.3 | 40 | 15.8 |
| | 11 | 120 | 19.0 | 40 | 21.1 |
| Dipterex | 132 | 260 | 96.9 | 160 | 98.7 |
| | 89 | 440 | 88.1 | 40 | 94.7 |
| | 44 | 90 | 71.0 | —[b] | —[b] |
| Malathion | 1,102 | 120 | 35.1 | 40 | 78.8 |
| | 440 | 120 | 14.7 | 120 | 12.5 |
| | 220 | 120 | 7.8 | 40 | 2.6 |
| Phenothiazine | 11,023 | 240 | 51.3 | 110 | 45.3 |
| | 6,613 | 120 | 51.7 | 40 | 40.3 |
| Ronnel | 220 | 120 | 97.2 | 40 | 97.0 |
| | 176 | 120 | 85.1 | 40 | 97.0 |
| | 132 | 120 | 88.7 | 120 | 85.7 |
| | 89 | 240 | 76.7 | 150 | 68.1 |
| | 22 | 120 | 29.4 | 40 | 7.9 |
| Dow ET-15 | 132 | 240 | 99.0 | 160 | 98.7 |
| | 89 | 240 | 97.0 | 160 | 98.7 |
| | 22 | 120 | 9.5 | 40 | 5.3 |
| | 11 | 120 | 9.5 | 40 | 0 |

[a] Concentrations in terms of technical insecticide.
[b] This concentration was omitted from the chick experiment.

The number of replications of droppings from each insecticide concentration tested ranged from a minimum of 4 to a maximum of 44. The untreated controls in each test consisted of droppings from the basal groups on insecticide-free diets.

EXPERIMENTAL RESULTS AND DISCUSSION.—The toxicity of the droppings to the 1-day-old house fly larvae is summarized in table 1. All larval mortality data were corrected for natural mortality by the use of Abbott's formula (Abbott 1925). Mortality of house fly larvae in the manure from the untreated control animals was low, ranging from 3% to 17%. Relatively low levels of Co-Ral, Diazinon, Dipterex, ronnel, and Dow ET-15 in the feed were highly toxic to the larvae. In all instances, dosages were changed during the course of the experiment in order to find a dosage that would be toxic to all larvae placed in the manure. This became impractical with malathion and phenothiazine which caused relatively low mortality to the house fly larvae at concentrations considerably higher than the other materials. Phenothiazine, in particular, caused low mortality in the house fly larvae.

There was no apparent difference in the effect on the house fly maggots between manure from the younger or the older animals on diets containing the same concentration of any insecticide except malathion. Manure from the chicks on a diet of malathion at 1,102 p.p.m. in the feed appeared to be more toxic to the larvae than the manure from the hens on this same diet.

The insecticide concentrations in the feed required to cause 50% mortality in the house fly larvae were calculated from the results of the tests utilizing the hen droppings. The L.C.-50's in terms of p.p.m. follow:

Co-Ral, 22; Dipterex, 28 (by extrapolation); Dow ET-15, 38; Diazinon, 47; ronnel, 48; malathion, >1,102; and phenothiazine, between 6,000 and 11,023.

The effect of these insecticides on the chickens with special emphasis on chick growth, weight gains, feed consumption, egg production, and chronic toxicity is discussed in detail by Ross & Sherman (in press). Their results showed that the treatments with ronnel or Dow ET-15 caused no observable deleterious effects in the birds or on egg production. All other insecticides caused some mortality or subclinical toxicity which was expressed as poor growth or decreased egg production.

Other workers have also recently studied the effect of the addition of insecticides to poultry feed for the control of house fly larvae. Quisenberry *et al.* (1958), Burns *et al.* (1959), and Sherwood (1959) investigated the larvicidal properties of droppings from chickens on rations containing Polybor 3 ($Na_2B_8O_{13}.4H_2O$). Levels ranging from 2,000 to 3,000 p.p.m. gave effective fly control. However, these same levels had deleterious effects on egg production.

Quisenberry *et al.* (1958) also reported that Trolene (ronnel) at levels as high as 535 p.p.m. in the feed did not control house fly larvae. This conclusion is at variance with the results reported here, which showed that ronnel at 220 p.p.m. caused 97% larval mortality.

The method used by Quisenberry *et al.* (1958) for determining the larvicidal effects of insecticide-containing manures has certain shortcomings. Their method allowed for the volunteer deposition of unknown quantities of house fly eggs with the subsequent determination of adult emergence. In such a method the control figures are purely relative and have no foundation on a known population

**Table 2.—Adult development of house fly larvae in manure from chickens on insecticide-treated rations.**

| Insecticide | Concentration[a] in Feed Mixture (p.p.m.) | Number of Larvae | | Survivors Becoming Adults (%) |
|---|---|---|---|---|
| | | Introduced | Surviving 3 Days After Treatment | |
| Co-Ral | 89 | 80 | 14 | 50 |
| | 44 | 80 | 28 | 43 |
| Diazinon | 89 | 70 | 33 | 76 |
| | 44 | 70 | 60 | 80 |
| Dipterex | 132 | 80 | 3 | 0 |
| | 89 | 40 | 9 | 89 |
| Malathion | 440 | 80 | 73 | 73 |
| Phenothiazine | 11,023 | 80 | 44 | 66 |
| Ronnel | 132 | 80 | 20 | 70 |
| | 89 | 80 | 16 | 50 |
| Dow ET-15 | 132 | 80 | 0 | — |
| | 89 | 80 | 5 | 100 |
| Untreated | — | 80 | 75 | 63 |

[a] Concentrations in terms of technical insecticide.

base. In the method described in this paper, where a known number of first-instar larvae are placed in the manure sample, it is possible to determine more accurately the statistics of mortality or control.

The relatively large volume of manure for each group of 10 individual larvae with the subsequent maintenance of sufficient moisture allows for rapid growth, so that in 3 days the unaffected larvae are well within the third instar and are easily detected by spreading the manure on paper toweling with a spatula. This 3-day period also provides ample time for the toxic effects of slow-acting insecticides to be exhibited.

Since it was desirable to know whether the surviving fly larvae had suffered any subclinical effects from the treatments that might be later expressed as failure to emerge, an experiment was conducted to study this possibility. On 2 separate days, while the regular mortality readings were being taken, the survivors in each of the four replicate jars were pooled without food and the number of adult flies formed in each group was noted. Table 2 summarizes the results of this experiment which show that the survival rate among the larvae from the insecticide-containing manures was at least as good as that among the larvae in the manure from the untreated controls. The low percentage of adult development was probably owing to nutritional deficiency caused by the premature removal of the larval food.

The results reported in this paper as well as those of Ross & Sherman (in press) indicate the possibility of using the chicken as a vehicle for distributing insecticidal materials in manure for the control of house fly larvae. Further studies, however, are necessary to determine the presence of toxic residues in poultry tissues and eggs.

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# Use of an Animal Membrane in the Evaluation of Chemical Repellents against the Stable Fly[1]

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ABSTRACT

Candidate insect repellents can be applied in laboratory tests to an animal membrane commercially available under the to an ox caecum membrane commercially available under the trade name Silverlight (derived from ox caecum; available from Julius Schmid, Inc., New York City). Repellency was determined by observing the number of caged stable flies (*Stomoxys calcitrans* (L.)) that fed or were prevented from feeding when the untreated side of the membrane was in contact with warmed beef blood. A count of the flies that have fed is facilitated by the use of an ultraviolet activated fluorescent dye previously added to the blood.

When results with this procedure were compared with those of another method in which chemicals were applied to cloth, it was found that the degree of repellency might vary depending on the surface to which the chemical had been applied; evaporation from the cloth or better retention on the membrane may account for some of this variation.

Evaluation by means of the membrane test method was found to more closely parallel the order of effectiveness obtained in field experience with repellents on dairy animals, and it may have these other advantages as well: ease and accuracy of counting fed and unfed flies; maintenance of uniform or variable applications and surface conditions; use of small amounts of chemicals; elimination of maintenance of an animal as an attractant; possible addition of chemicals to the blood for systemic repellency or other types of testing; and the possible use of liquid media other than blood for attracting flies to the treated membrane.

Various means and procedures for feeding and rearing arthropods on animal-derived membranes have been indicated by Tarshis (1958a and 1958b). Bar-Zeev & Smith (1959) utilized one of these membranes to study the action of mosquito repellents and found it a satisfactory surface for use in evaluating the effectiveness of chemicals against *Aedes aegypti* (L.). In their studies the repellents were applied to the membrane at various concentrations, and thus made it possible to determine the concentration required to give 50% repellency. In the method described by Bar-Zeev & Smith a chemical was applied to an animal membrane (Silverlight[2]) that formed the bottom portion of a dish containing blood. When the mosquito cage was brought in contact with the membrane, the mosquitoes probed through from below.

Results of initial attempts in this laboratory to assess the feasibility of using a similar procedure with stable

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[2] Derived from ox caecum; available from Julius Schmid, Inc., New York City.

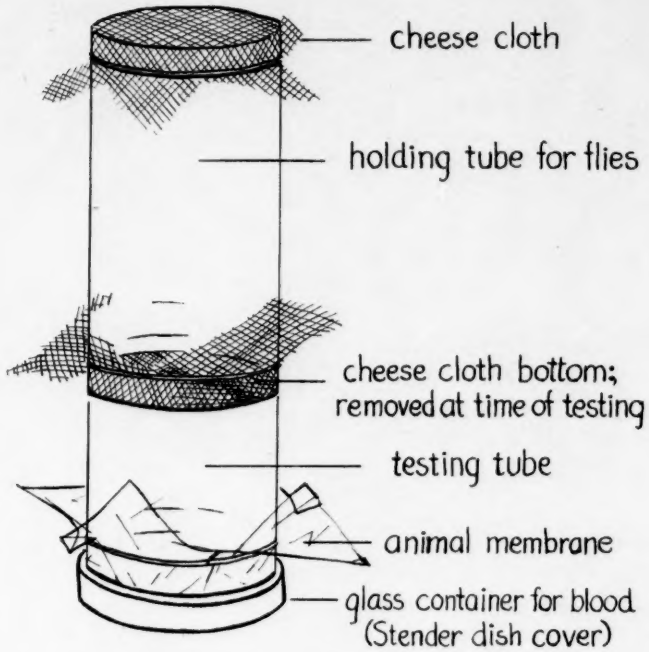


FIG. 1. Apparatus in position for testing repellents.

flies (*Stomoxys calcitrans* (L.)) were unsatisfactory. The probing of the flies was frequently found to cause seepage of blood through the membrane, thus allowing the flies to feed on the droplets of blood suspended on the surface of the membrane and making it unnecessary for them to penetrate the treatment. A considerable number of attempts were made to overcome this difficulty. Thickening or gelling agents, such as filter cel and a water-soluble resin, were added to the blood, but the flies had difficulty obtaining enough liquid blood from the media even when the membranes were not treated with repellents. A similar result occurred when the blood was soaked on cloth or spongelike substances placed on the inner side of the membrane. Other animal-derived membranes and synthetic films ("Nearkid," "Zephyr," rubber dental dam, polyethylene film) either restricted feeding or were similar to the Silverlight product from the standpoint of seepage.

The difficulty was finally overcome when the membrane was used to cover a dish full of blood (instead of being used as the bottom of the dish) and the fly cage placed *over* the dish so that the flies rested on top of the membrane. When care was taken to select a membrane free of obvious holes, the weight of the blood no longer forced it through openings made by the feeding of the flies. The following description presents the details of this procedure for the evaluation of chemicals for repellency to stable flies.

PROCEDURES.—A 4-by-4-inch piece of Silverlight membrane was fastened with a rubber band on one end of a Lucite tube 2 inches in diameter by 2.5 inches in length. Two-tenths milliliter of an acetone solution of a candidate repellent was spread with a pipette on the side of the membrane which faced the inner side of the tube. This treatment was air dried 20 to 24 hours in a fan-ventilated cabinet.

Stable flies 4 to 8 days old were used for the repellency tests. They were immobilized with carbon dioxide 6 to 18 hours prior to use and 20 to 40 flies were transferred from the rearing cage to a holding cage, a Lucite tube, 2 inches in diameter by 5 inches in length, with cheesecloth fastened to the ends with rubber bands. Normally when tests were made in the morning, the flies were placed in the holding tubes in the late afternoon of the previous day, at which time the cheesecloth on one end of the tube was moistened with water. This method permitted ample time for the flies to recover from the effects of the carbon dioxide and starved them sufficiently to make them avid for blood.

At the time of testing citrated beef blood was warmed to 100° to 110° F., and 5 ml. was pipetted into the depressed portion of a stender dish cover. The tube with the attached membrane was then placed with the untreated side touching the blood. The inner surface of the membrane consequently became warmed to approximately 90° F.

Immediately after the treated membrane had been placed on the warmed blood, a tube containing the starved stable flies was placed in contact with the open end of the tube containing the membrane. The intervening cheesecloth on the tube containing the flies was then withdrawn permitting the flies, if they were not repelled by the treatment, to have access to the blood. A diagrammatic sketch of the testing apparatus is shown in figure 1.

After a 10-minute exposure both tubes were removed from the blood; the flies were immobilized with carbon dioxide and a count was made of those that did not feed.

**Table 1.—Repellency of chemicals on animal membranes or cheesecloth exposed to stable flies in the laboratory.**

| Treatment[a] | No. of Replicates | Average Per Cent Repellency[b] at Concentrations of (%) | | | | RC[c] 50% |
|---|---|---|---|---|---|---|
| | | 1.25 | 2.5 | 5.0 | 10 | |
| *When on membranes* | | | | | | |
| Butoxy polypropylene glycol | 15 | — | 48.6±17.6 | 71.4±13.1 | 85.8±14.4 | 2.6 |
| Delphene | 12 | — | 13.7± 9.6 | 32.0±25.6 | 86.6±10.2 | 5.4 |
| Indalone | 15 | 6.5± 9.1 | 12.4±10.2 | 71.2±27.1 | — | 4.5 |
| MGK R-326 | 12 | 34.6±17.9 | 79.6±11.9 | 90.2± 7.2 | — | 1.7 |
| Tabatrex | 12 | — | 5.2± 5.6 | 8.7± 7.2 | 21.9± 9.9 | >10% |
| *When on cheesecloth* | | | | | | |
| Butoxy polypropylene glycol | 15 | — | 22.2±12.8 | 30.5±15.5 | 43.9±12.6 | >10% |
| Delphene | 12 | — | 11.7± 6.6 | 31.6±27 | 80.6±17.6 | 6.4% |
| Indalone | 15 | 26.9±24.4 | 70.9±30.9 | 88.9±21.8 | — | 2.1% |
| MGK R-326 | 12 | 5.8± 5.8 | 23.4±16.4 | 39.5±12.1 | — | > 5% |
| Tabatrex | 12 | — | 5.6±17.1 | 18.8±11.3 | 41.9±15.6 | >10% |

[a] For 15 replicates of acetone check treatments on membranes there were 6.3± 5.1% unfed flies; on cheesecloth there were 5.9± 6.5% unfed flies.
[b] Standard deviation of the mean is shown for each figure.
[c] Concentration at which 50% of the flies were repelled.

Per cent repellency was determined by comparison with the total number exposed.[3]

As a check on the activity of each batch of flies used, a membrane treated with 0.2 ml. acetone was used with each series of replicated tests. When some of the flies failed to feed in this check treatment, Abbott's formula[4] was used to correct the per cent repellency of the results obtained with the treatments.

Determination of feeding was facilitated if a fluorescent chemical[5] was previously mixed with the blood. When the flies under carbon dioxide anesthesia were illuminated with ultraviolet black light,[6] the abdomens of the flies that had fed on blood in which the dye was incorporated fluoresced a bright green while those that had not fed remained black. In ordinary light many of the abdomens of the flies that had fed were red and distended. Those that had fed only partially, however, were difficult to distinguish from unfed flies. The use of the fluorescent pigment and ultraviolet light eliminated this uncertainty and has been routinely used in all the tests at the rate of 0.5% of the dye mixed into the warmed blood. No adverse effect on the flies was noted from the use of the dye.

Comparison of this procedure has been made with one in which the chemical was applied to cheesecloth (Starnes & Granett 1953). A piece of 6×12 inch cheesecloth was impregnated with 7.5 ml. of an acetone solution and then air dried 24 hours in a fan-ventilated cabinet. At the time of testing one of the cheesecloth ends was removed from the Lucite holding cage and replaced with a piece cut from the treated cloth. The untreated cloth was then put back over the treated cloth. The end of the cage with this double layer was placed on the belly of a shaven rabbit. The untreated layer rested against the rabbit (to prevent contamination of the skin by the chemical), while the treated cloth was exposed to contact of the flies as they attempted to feed on the rabbit. Repellency was judged by comparing the numbers that did not feed with the total numbers in the tube. Separate tubes were used for replicate tests.

RESULTS.—To illustrate the results that might be obtained, five recognized repellent substances—butoxy polypropylene glycol (Crag Fly Repellent); Delphene® (N,N-diethyl-*m*-toluamide); Indalone® (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate); MGK R-326 (di-*n*-propyl isocinchomeronate); and Tabatrex® (dibutyl succinate) were applied at three concentrations to Silverlight membranes and to cheesecloth using the procedures already described. Replicate tests were made over a period of several months. The per cent repellency is indicated in table 1 as well as the RC50, the concentration at which 50% of the flies were repelled as determined from eye-fitted lines on log-probit paper.

It is apparent from these results that variation in repellency can be detected; the degree of effectiveness appears to depend somewhat on the surface to which the chemical was applied. Butoxy polypropylene glycol, for instance, had an RC50 of 2.6% when on membranes, whereas on cheesecloth the RC50 for this chemical was greater than 10%. Indalone, on the other hand, had an RC50 of 5.4% on membranes but was more repellent on cloth, having an RC50 on the cloth surface of 2.1%. An even more startling difference occurred with MGK R-326, which had an RC50 of 1.7% on membranes but was relatively poor against stable flies when on cloth, having an RC50 of more than 5%. The superior repellency of butoxy polypropylene glycol, MGK R-326, and to a lesser extent Delphene when on membranes, cannot be attributed to the quantity of chemical deposited on the surfaces. If a 5% acetone solution were used on the 2-inch diameter circles of the membranes, it would amount to 0.002 ml. chemical per square inch, and on the saturated cloth it would be 0.005 ml. chemical per square inch. It is possible that certain of the chemicals are more readily retained by the animal membrane than the cloth. A factor favoring quicker dissipation from cloth is its porous character, which permits greater opportunity for evapo-

[3] $$\text{Per cent repellency} = \frac{\text{Number not feeding}}{\text{Total number exposed}} \times 100$$

[4] Corrected per cent repellency

$$= \frac{\%\text{ repellency treated} - \%\text{ repellency acetone check}}{100 - \%\text{ repellency acetone check}}$$

[5] Blancophor HS76, Antara Chemicals, General Aniline & Film Co., New York, N. Y.

[6] A Sylvania 6-watt Blacklight Blue bulb was used but other types providing similar illumination are satisfactory.

Table 2.—Repellency of oil base sprays applied to dairy cattle[a] exposed to biting flies in the pasture.

| TREATMENT[b] | AVERAGE PER CENT REPELLENCY OF BITING FLIES[c] AFTER HOURS SHOWN | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 28 |
| Butoxy polypropylene glycol | 85 | 82 | 82 | 58 |
| Delphene | 83 | 62 | 70 | 32 |
| MGK R-326 plus MGK 264[d] | 85 | 62 | 51 | 51 |
| Tabatrex | 67 | 41 | 46 | 20 |

[a] Brown Swiss and Jersey breeds. Animals were observed for susceptibility to fly attack prior to spraying and separated into groups of five of approximately equal susceptibility. Sprays were applied to 5 animals 3 times at 4- to 5-day intervals.

[b] Two ounces of 5% concentration in Bayol D petroleum oil were sprayed on each animal except that MGK 326 was used at 2% plus 2% MGK 264 synergist

[c] Horn flies and stable flies. Fly counts made at the periods indicated following each treatment.

$$\text{Per cent repellency} = \frac{\text{No. flies on untreated—No. on treated}}{\text{No. on untreated}} \times 100$$

[d] *N*-(2-ethylhexyl)bicyclo [2.2.1]hept-5-ene-2,3-dicarboximide.

ration during the 24-hour exposure period prior to testing. In any event this comparison tends to indicate that the nature of the surface may have an unpredictable effect on repellency. Tabatrex, known to be effective against house flies (*Musca domestica* L.) had a relatively poor effect against stable flies by both methods tested.

In a few instances, at one or another concentration level, the standard deviation from the mean may appear large. While considerable variation is not unusual in screening chemicals for insect repellency, if only one concentration of a chemical were tested a large variation or a low repellency figure might result in discarding a chemical that could have potential value. Use of several concentrations, determination of an RC50 figure, and use of a comparison compound of known repellency tend to reduce this possibility.

The factors influencing repellency in the field are probably more complex than those in the laboratory. Field tests with four of the chemicals against biting flies (horn flies (*Siphona irritans* (*L.*)) and stable flies) annoying dairy cattle showed, however, a closer agreement with the laboratory tests on membranes than those on cheesecloth. Table 2 presents the average repellency observed on dairy cattle in 1958 for three periods following spraying and on the following day.

In these tests butoxy polypropylene glycol appeared to be the most persistent of the chemicals. MGK R-326, however, had been tried at 2% plus 2% synergist MGK R-264 instead of being used at 5%. The MGK R-326 formulation used was approximately equivalent in effectiveness to Delphene on the day sprayed and was somewhat better than the latter compound on the following day. As in the laboratory tests, Tabatrex was least effective against the biting flies.

Indalone was not included in this series. Tests reported in 1953 (Starnes & Granett) showed this compound to be relatively ineffective against biting flies in the field as compared with butoxy polypropylene glycol. These results also correspond more closely with those obtained on membranes than on cheesecloth.

In addition to a possible closer approximation to field experience, when repellents are applied to animals, some of the advantages that might be listed for the use of the membrane method for testing repellents are:

Fed and unfed flies can be counted accurately and rapidly when an ultraviolet light activated fluorescent dye is used in the blood.

Temperature, humidity, and other conditions of treated surface and attractant (blood) can be varied or maintained readily.

The surface is more similar to that of animal skin on which the repellents are most likely to be applied, than does cloth.

The amount applied can be adjusted more readily than on cloth.

There is no need to maintain animals for use in testing.

Only a small amount of chemical is needed for each replicate.

Chemicals may be added directly to the blood used as attractant. This method may be useful for the study of systemic-type repellents or for other types of physiological or toxicological testing.

Liquid media other than blood may be used to attract the flies to the treated membrane. Stable flies have fed readily on sugar solution in which the fluorescent dye had been incorporated.

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## NEW ADDRESS

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# Stalk Shredding and Plowing as Methods for Controlling the Pink Bollworm, *Pectinophora gossypiella*[1]

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ABSTRACT

Several types of stalk shredding machines were tested for efficiency in destroying field populations of pink bollworm (*Pectinophora gossypiella* (Saund.)) larvae. Kills of up to 88% of the larvae were obtained, depending on type of machine used. Best results over all tests were obtained with the flail chopper. The conventional rotary stalk shredders were the least efficient. Plowing tests and winter burial studies indicated that 76% to 83% of the pink bollworm larvae in infested cotton debris were killed by burying the material during the winter. Survival of pink bollworms in infested cotton left over winter on the soil surface averaged 51.6%. These data emphasize the importance of and the benefits that may be obtained by a thorough job of stalk destruction.

Community-wide stalk destruction was included among the earliest practices recommended for control of the pink bollworm (*Pectinophora gossypiella* (Saund.)). One of the objectives of this practice is the destruction of overwintering larvae, most of which are found in infested bolls remaining in the field after harvest. In addition, in certain areas, stalk destruction also provides a host-free period which serves to break the reproductive cycle of the insect, and in some cases, may reduce the potential numbers of overwintering larvae by eliminating the food supply before frost. Until recent years, stalk destruction was accomplished by cutting the stalks by hand, then raking and burning them. Stalk destruction, like other farm practices, has now become mechanized and improved stalk destruction machines are continually being developed. The Texas Station has undertaken the evaluation of these machines for efficiency in destroying pink bollworm larvae left in infested bolls remaining in fields after harvest.

A preliminary report of this work published by Adkisson *et al.* (1958) indicated that the results were subject to variation depending on the design of the machine used. These authors reported that over a three-year period, an average of 55.2% of the pink bollworms in infested bolls left in the field after harvest were killed by shredding with a conventional rotary shredder. The latter type is the one most commonly used by farmers in Texas. Kills up to 97.5% of the boll populations of larvae were obtained by the use of ensilage harvesters having a flail chopper design. These results indicated that the field population of pink bollworms may be drastically reduced by stalk shredding, and that certain types of machines are more effective than others.

Experimental results from many tests have shown that plowing under or burying of infested crop remnants will result in high mortality among the larvae residing in the debris (Loftin *et al.* 1921, Ohlendorf 1926, Fenton & Owen 1953, Noble 1955, Fife *et al.* 1957). However, as pointed out by Fenton & Owen (1931) soil moisture appears to be an important factor in achieving best results by plowing as these workers reported that larvae in cotton bolls buried 4 inches in dry soil had a higher survival than those left on the soil surface. However, when the buried material was irrigated, the mortality of the larvae was higher than that recorded for the insects left on the soil surface. The reports reviewed indicated that highest mortalities by plowing could be achieved when winter environmental conditions were unfavorable for larval survival. The two conditions mentioned as being most detrimental to the hibernating pink bollworms were high soil moisture conditions and cold temperatures. Fife *et al.* (1957) presented data taken over a 4-year period in Central Texas that indicated 98% mortality of pink bollworms in infested material buried 2 inches during November as compared with 73% mortality occurring in infested cotton left on the soil surface. Results of the work reviewed indicated that a stalk destruction program consisting of shredding and plowing under of the infested crop remnants should be very effective for reducing winter populations of the pink bollworm in Central Texas.

STALK SHREDDER TESTS.—Three small-plot field experiments were conducted in Central Texas during 1958 to evaluate the relative efficiency of several types of machines for reducing pink bollworm larval populations left in infested fields after harvest. Two of the experiments were conducted near Waco and one near College Station. Machines tested in the three experiments included a conventional rotary type stalk shredder, a modified conventional rotary shredder, a flail chopper and a utility chopper, with and without modification. The flail chopper and utility chopper are most commonly used as ensilage harvesters.

The conventional rotary stalk shredder has a single rigid blade which rotates on a vertical shaft.

The modified conventional rotary stalk shredder was equipped with a special cutting blade. The modification was made by welding a V-shaped cup-like attachment on each tip of the cutter blade. During operation of the machine, these cups created an air blast. The design was based on the theory that the air blast would be sufficient to pick up any infested cotton on the ground and which would cause this material to be exposed to the action of the cutting blade.

The flail chopper has a horizontal shaft on which 40 vertical, free swinging knives are mounted.

The utility chopper has a vertical shaft equipped with four blades. The blades are provided with V-shaped attachments on the tips. This attachment creates considerable suction when the blades are rotating.

For one series of treatments, the utility chopper was modified by attaching a pair of corrugated 6-inch steel crusher rolls to the discharge opening. The shredded material was passed through these rollers as it was being discharged from the machine. In theory, these rollers

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Table 1.—Performance data of machines used in cotton stalk shredder tests, 1958.

| TYPE OF MACHINE | TYPE OF BLADES | SHAFT SPEED (R.P.M.) | BLADE TIP SPEED (F.P.M.)[a] | WIDTH OF SWATH (INCHES) | GROUND SPEED (M.P.H.) |
|---|---|---|---|---|---|
| Conventional rotary | Straight rigid | 810 | 13,350 | 63 | 1.7 |
| Modified rotary | Modified-lift rigid | 810 | 13,350 | 63 | 1.7 |
| Flail chopper | Free-swinging curve | 1,620 | 11,000 | 80 | 1.7 |
| Flail chopper | Free-swinging curve | 1,620 | 11,000 | 80 | 2.5 |
| Utility chopper | Modified-life rigid | 675 | 11,664 | 66 | 1.7 |

[a] Feet per minute.

should have crushed any pink bollworms in this material.

*Experiment 1.*—The experiment was conducted near Waco in a field just after the cotton had been harvested. Crop residue consisted mainly of unopened green bolls or insect-damaged bolls which had been left in the field after the crop had been hand harvested. Stalks were approximately 3½ feet in height.

The test was designed as a randomized block with treatments replicated four times. Plots were four rows wide and 50 feet long. Machines tested included the conventional rotary stalk shredder, the modified rotary stalk shredder, and the flail chopper. Operational data for the machines are given in table 1. In this test, the flail chopper was operated only at 1.7 m.p.h.

Records were made of the number of unopened green bolls on 25 feet of each of the two center rows in each plot before and after shredding. Only green bolls which apparently were not damaged or only slightly damaged, but with the carpels intact, were counted after shredding. Twenty-five unopened green bolls were randomly collected from each plot before shredding for larval counts. An after-shredding sample of a like number consisting of unopened green bolls that apparently were not damaged or that were only slightly damaged with the carpel intact were collected from the plots. These bolls were taken to the laboratory, carefully dissected by hand, and examined for pink bollworm larvae. The efficiency of the machines was compared on the basis of the number of larvae in the green bolls in the before- and after-shredding samples.

Results are presented in table 2. The flail chopper proved much more efficient in destroying green bolls than the other two machines. This machine destroyed 71% of the green bolls compared with 21% and 26% for the modified rotary and conventional rotary shredders, respectively. The modified life type blades did not increase the effectiveness of the conventional rotary shredder as the blades apparently did not develop enough suction to pick up the green boll material present on the soil surface.

Table 2.—Relative efficiencies of certain stalk shredders for destroying green bolls and for reducing pink bollworm larval populations in green bolls.

| CRITERION | MACHINE | | |
|---|---|---|---|
| | Conventional Rotary | Modified Rotary | Flail Chopper |
| Green bolls before shredding[a] | 75 | 56 | 63 |
| Green bolls after shredding[a] | 55 | 44 | 18 |
| Per cent destroyed | 26.7 | 21.4 | 71.4** |
| Larvae per boll before shredding | 4.7 | 4.0 | 4.6 |
| Larvae per boll after shredding | 3.1 | 2.9 | 2.4 |
| Per cent reduction | 34.0 | 27.5 | 47.8 |

[a] Average number of green bolls per 50 feet of row.
** Significantly different from the other two machines at the 1% level.

The overall average pink bollworm infestation before shredding was 4.4 larvae per boll. Results indicated that the flail chopper was slightly more effective than the other machines in reducing the pink bollworm population in the apparently undamaged or slightly damaged green bolls. These results are presented in table 2. If it can be assumed that all the pink bollworms were killed in the 71% of green bolls destroyed by the flail chopper; this, plus the 48% that were killed in the slightly damaged bolls would indicate that the machine was very effective in decreasing the total overwintering larval population found in green bolls left in the field after harvest. It is interesting to note that all of the shredding machines killed a certain percentage of the larvae in the apparently undamaged or slightly damaged green bolls. This kill probably resulted from impact in the process of passing through the machine in much the same manner as larvae killed in the ginning process as reported by Robertson *et al.* (1959).

*Experiments 2 and 3.*—Experiment 2 was conducted in the same field as experiment 1 but about 10 days later. The cotton had been desiccated previously and most all of the bolls were open at the time of shredding. Crop residue consisted mainly of dry stalk material and partially opened bolls left in the field after the crop had been hand picked. Experiment 3 was located in a field near College Station. This experiment was conducted after frost. Crop remnants consisted mainly of standing stalks and partially opened bolls which were left after mechanical picking of the crop. Some scattered boll and seed material was also on the ground. Stalks were approximately 4 feet high at the time of shredding.

The same experimental procedure was followed in both tests with the exception of the type of machines used as indicated in table 3. Plot size and design were the same as outlined for experiment 1. Unshredded check plots were included among the treatments for comparison.

Pink bollworm records were obtained from the number of moths emerging from the shredded and unshredded residues. In the check plots, all the seed cotton and boll residues were gleaned from the stalk and picked up from the ground over an area of 1/250 acre. A sampling frame encompassing an area this size was used for sampling all the plots. The frame was randomly placed toward the center of each plot and all seed cotton and boll material encompassed by this frame were collected. Samples were taken from the machine plots after shredding and in-

**Table 3.—Relative efficiencies of several types of stalk shredders for pink bollworm control, Waco and College Station, Texas. 1958.**

| Machine | Waco, Expt. 2 | | College Station, Expt. 3 | |
|---|---|---|---|---|
| | Moth Emergence per Acre | Per Cent Reduction | Moth Emergence per Acre | Per Cent Reduction |
| Unshredded check | 16,563 | — | 18,750 | — |
| Conventional rotary | 10,063 | 39 | — | — |
| Modified rotary | 6,438 | 61 | 5,688 | 70 |
| Flail chopper, 1.7 m.p.h. | 3,063 | 82 | — | 88 |
| Flail chopper, 2.5 m.p.h. | 4,437 | 73 | 2,250 | — |
| Utility chopper | — | | 2,625 | 86 |
| Modified utility chopper | — | | 4,750 | 76 |
| L.S.D. at 5% level | 4,875 | | 6,443 | |

cluded the shredded material and any waste cotton that may have been on the ground prior to shredding.

The collected crop material was placed in 5-gallon tins fitted with small glass collection tubes. The tins were fitted with screened openings in the tops to permit ventilation. The cans were stored in a constant temperature room maintained at 80° F. ± 5°. Daily records were made until moth emergence had ceased.

Results are presented in table 3. In the Waco test, all the shredders produced significant reductions in number of pink bollworms when compared with the check. The percentage reduction ranged from 39% to 73%. The flail chopper produced a significantly greater kill of pink bollworms than the conventional rotary shredder. The blade modification of the rotary shredder appeared to increase its efficiency.

Results of the College Station test indicated no significant differences among machines. All the machines significantly reduced moth emergence when compared with the check. However, the flail chopper appeared to be slightly more efficient than the modified rotary and about equal to the utility chopper. Modification of the utility chopper by the addition of the crusher rolls did not improve the performance of this machine. Percentage reductions of pink bollworms ranged from 70% for the modified rotary shredder to 88% for the flail chopper.

The efficiency of the flail chopper was attributed to: (1) the tremendous shredding action of this machine which cut the crop remnants into much smaller pieces than the other machines did, (2) the air blast or suction created by the machine which picked up waste cotton on the ground before shredding and passed it through the shredding machine, and (3) the great force created by the knives that hurled much of the crop material against the sides of the machine and probably killed some larvae by impact.

Results of the shredder experiments indicated that considerable reduction in the pink bollworm population can be made by shredding cotton stalks after harvest. The relative amount of mortality obtained will depend on field conditions and type of machine used. These tests indicated that highest mortality of pink bollworms may be obtained with the flail chopper or the utility chopper. Records covering 2 years of previous testing of the flail chopper (Adkisson *et al.* 1958) together with records from the experiments cited above indicated that reduction in pink bollworm populations in infested crop remnants ranging from 73% to 97% may be expected by using this type of machine. The average reduction in all tests with the flail chopper during this 3-year period was 87.6%. The percentage kill obtained with this machine will depend in part on field conditions such as stalk size, dryness of material and amount of infested material on the soil surface before shredding.

Plowing Test.—Moldboard and listertype plowing was compared for effectiveness in reducing pink bollworm field populations in a randomized block, small plot experiment. Each plow treatment and an unplowed check were replicated four times. Five pounds of pink bollworm infested cotton was placed in two square-yard areas in each plot. The material was turned under in the plow treatments. The moldboard plow turned the infested material under to depths of approximately 4 to 6 inches and the lister threw it up into beds. The untreated check material was left on the soil surface. Immediately after each plot was plowed, the area containing the infested material was confined in screen pyramidal cages. One cage covered one-half of the 5-pound sample of infested cotton, making a total of two cages per plot. The cages were fitted with collection tubes for trapping the pink bollworm moths as they emerged. The test was installed January 29, 1959, and after plowing was accomplished, the soil was not reworked until after records of moth emergence were completed.

Daily records of moth emergence were taken beginning with the appearance of the first moth and continued until emergence ceased. The number of moths collected per plot are reported in terms of moths per acre. The efficiency of the plows was rated according to number of moths that emerged in the plowed plots in comparison with the number recorded from the unplowed checks.

Results are given in table 4. There were no significant differences between plows in reducing moth emergence. Both plows gave highly significant reductions in pink bollworm populations over that recorded for the check. Results indicated more than 80% reduction of the field pink bollworm larval population as a result of plowing.

In conjunction with the plowing test, a study was made of the effects of winter burial of infested cotton on survival of the hibernating larvae. Five pounds of infested cotton harvested the previous fall were installed in each of 24 one-square-yard pyramidal cages on February 4, 1959. The cotton in 12 cages was buried approximately 2 inches deep and in the other 12 it was left on the soil surface. Treatments were arranged in a completely random order. The cages were fitted with collection tubes and daily moth emergence was recorded.

Results indicated an average of 22.4 moths per cage emerged from the infested bolls left on the soil surface as compared with 5.4 moths per cage of buried material. Moth emergence was reduced 78.5% as the result of

**Table 4.—Results of plowing tests for pink bollworm control, College Station, Texas. 1958.**

| Treatment | Moth Emergence per Acre | Per Cent Reduction |
|---|---|---|
| Unplowed check | 17,545 | — |
| Moldboard | 3,025* | 83 |
| Lister | 3,340* | 81 |

* Significantly different from the check at the 1% level of probability.

burial. This reduction was significant at the 1% level of probability. Results were comparable to those obtained in the plowing test.

DISCUSSION.—Data presented for stalk destruction equipment indicated that approximately 85% to 90% of the pink bollworm larvae left in the field in postharvest crop remnants may be killed by shredding with the most efficient machine. Results of plowing and winter burial experiments showed that 75% to 80% of the pink bollworms in infested cotton could be killed by winter burial under conditions existing in Central Texas.

A review of the records of emergence of overwintered pink bollworms at College Station for the period 1954–59 indicated survival of moths in infested bolls held on the soil surface during the winter months averaged 51.6% (Brazzel & Martin 1959, Williams 1959, unpublished data, 1960). Survival ranged from a maximum of 93.4% in the winter of 1954–55 to a minimum of 9.2% in the winter of 1956–57.

These data combined emphasize the importance of a community-wide stalk shredding and plowing program soon after the harvest in infested areas is completed. In Central Texas, a community program of stalk shredding followed by plowing under of crop residue plus natural winter mortality and a degree of suicidal emergence of moths in the spring before cotton fruit is present, should drastically reduce the threat of pink bollworm attack during the following year and might delay buildup of damaging numbers. When this program is coordinated with a program of early crop production practices such as a uniform planting period, timely insect control, early maturing varieties, early harvest, et cetera, it appears that in many years a cotton crop can be produced and harvested in Central Texas before pink bollworm populations can develop to economic proportions.

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# Soil Treatments in Lieu of Spraying for Plum Curculio Control in Peach Orchards[1]

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ABSTRACT

In soil-box experiments aldrin and dieldrin prevented emergence of adults of the plum curculio (*Conotrachelus nenuphar* Hbst.) for eight seasons, and heptachlor dust for seven and granules for four seasons. Chlordane granules were more effective than those of isodrin, endrin, or Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide). No differences due to formulation were found. A soil treatment with 2.3 pounds of aldrin per acre in a peach orchard in March 1957 held the percentage of harvested, infested peaches to 1.6 in 1957, 2.9 in 1958, and 0.7 in 1959 without the help of insecticides applied to the trees. Only small numbers of adults were jarred from the trees during those years. In another orchard 2 pounds of aldrin per acre prevented the development of adults from most of the larvae placed on the soil under the spread of sample trees over a 3-year period. Soil treatments with aldrin, dieldrin, or heptachlor are now being used as a supplement to spraying by a number of Georgia peach growers.

Chlorinated hydrocarbons have been under investigation as soil insecticides for the control of the plum curculio (*Conotrachelus nenuphar* Hbst.) at Fort Valley, Georgia, since 1952 (Snapp 1953). Screening experiments in soil boxes have been conducted every year, and during the last 3 years single applications of aldrin were made in peach orchards, including one in which no sprays were used. This paper reports the experiments conducted in the last three years. The earlier soil-box tests have already been reported.

SOIL-BOX EXPERIMENTS.—Wooden boxes holding 1 square yard of soil 6½ inches deep (Snapp 1939) were used in the screening experiments. Dusts were applied with a household sugar shaker or sieve, granules were broadcast by hand, and sprays were applied with a hand sprayer. They were immediately worked into the soil with a small rake for 5 minutes to a depth of 3 inches. Each treatment was applied to two boxes in 1952 and to one

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[2] Julius P. Hollon assisted with these experiments.

**Table 1.—Results of soil-box experiments with insecticides for the control of the plum curculio, 1957–59.**

| Date Treated | Insecticide (Pounds of Active Ingredient per Acre) | Per Cent Emerging as Adults | | |
|---|---|---|---|---|
| | | 1957 (200–250)[a] | 1958 (750) | 1959 (750) |
| May 2, 1952 | Aldrin dust | | | |
| | 2 | 7.6 | 0.1 | 0.2 |
| | 4 | 3.4 | 0.1 | 0.8 |
| | Dieldrin dust | | | |
| | 2 | 5.0 | 0.1 | 1.3 |
| | 4 | 2.4 | 0 | 0.4 |
| May 28, 1953 | Heptachlor dust 4 | 0 | 0 | 0 |
| June 29, 1953 | Isodrin dust 4 | 33.2 | | |
| April 12, 1954 | Aldrin granules 4 | 4.1 | 0 | 0.1 |
| | EC 4 | 0 | 0 | 0 |
| May 31, 1955 | Chlordane granules 4 | 5.5 | 4.0 | 0.8 |
| April 20, 1956 | Heptachlor granules 2 | 0.5 | 0 | 0 |
| April 8, 1958 | Endrin granules 2 | — | 18.1 | 17.6 |
| April 8, 1959 | Thiodan dust | | | |
| | 2 | — | — | 66.3 |
| | 5 | — | — | 18.3 |
| | Untreated | 55.8 | 51.2 | 59.2 |

[a] Numbers in parentheses indicate number of larvae per box.

box in subsequent years. The boxes were exposed in the open to the weather. Each year they were stocked with newly matured first-generation plum curculio larvae as soon as sufficient numbers became available. The effectiveness of the insecticides was measured by the adult emergence. The treatments were evaluated each year as long as they prevented adult emergence.

The results of these experiments are given in table 1. Aldrin and dieldrin dusts were effective for the eighth season and heptachlor dust for the seventh and granules for the fourth season. Chlordane was less effective than aldrin, dieldrin, or heptachlor, and isodrin, endrin, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) gave poor control. No differences owing to formulation were found.

Orchard Tests.—The soil under the spread of 441 Regular Elberta peach trees in an orchard near Reynolds, Georgia, was treated by hand with 2% aldrin granules on March 11 and 18, 1957, at the rate of 175 grams of granules per tree, equivalent to 2.3 pounds of aldrin per acre. The granules were worked into the soil on the same day by harrowing two ways with a double-section disk harrow or tool-bar scratcher. No insecticide was used on the trees during the seasons of 1957, 1958, and 1959. Groups of four trees replicated five times were selected at random before harvest from which to obtain records of curculio injury. The fruit from these trees was harvested at the same time as that from the remaining trees, and samples from each of the several pickings were cut and examined. Only 1.6% of the harvested peaches were infested in 1957, 2.9% in 1958, and 0.7% in 1959. Sample trees in the orchard were jarred several times during each growing season to determine the adult curculio population. Few adults were present at any time, the average number per tree never exceeding one. The beetles taken before July in 1957 matured before the aldrin was applied, and some of those taken subsequently may have migrated into the test orchard from outside hosts.

On January 3–5, 1956, the soil under 592 Pearson Elberta peach trees was treated with 2 pounds of aldrin per acre in granules that were worked into the soil with a double-section disk harrow immediately after application. The 561 trees in the remainder of the orchard were left untreated. Early in May 1957 bottomless 1-square-yard cages were sunk into the soil under the spread of two trees selected at random in the treated section of the orchard and under one tree in the untreated section. Fifty mature plum curculio larvae were placed on top of the soil in each cage on May 28, and the emerged adults were recorded and removed between June 25 and the last of July. Early in May 1958 and again late in June 1959 similar cages were placed under the spread of four trees selected at random each year in both the treated and untreated sections. Each cage was charged with 200 mature larvae during the period May 14–23 in 1958 and July 9–14 in 1959. The emerging adults were recorded and removed between June 13 and July 2 in 1958 and August 4 and 24 in 1959.

The effectiveness of the treatment is evident from the percentages of larvae that emerged as adults each year, as given below:

| | *Treated Section* | *Untreated Section* |
|---|---|---|
| | *1957* | |
| | 0 | 34.0 |
| | 0 | — |
| | *1958* | |
| | 2.0 | 48.5 |
| | 0.5 | 73.0 |
| | 3.0 | 72.0 |
| | 0 | 73.5 |
| Average | 1.4 | 66.8 |
| | *1959* | |
| | 10.5 | 79.0 |
| | 3.5 | 83.0 |
| | 10.0 | 82.0 |
| | 2.0 | 58.5 |
| Average | 6.0 | 75.6 |

These results indicate that a soil treatment may be used in lieu of spraying the trees to control the plum curculio in peach orchards.

Soil Treatment by Peach Growers.—A number of growers in Georgia treated the soil in their orchards with aldrin, dieldrin, or heptachlor as a supplement to spraying for the control of the pulm curculio. The first week in March 1958 the soil under 1350 Maygold, 3000 Red Cap, and 2000 Sullivan's Elberta trees in an orchard near Perry was treated with 2⅓ pounds per tree of a mixture prepared by adding 64 pounds of 25% aldrin to each ton of 10-10-15 fertilizer. This provided for the distribution of aldrin at the rate of 2 pounds per acre. The material was broadcast under the trees from tin cups in the hands of laborers.

The fruit on the Sullivan's Elberta trees was inspected on July 16, 1958, when about one-third of the crop had been harvested, and no plum curculio infestation was found. The owner had not seen a wormy peach from the orchard to that time. Since curculio-infested peaches are usually found in the early pickings, any infestation would have been evident by then. In 1959 the owner had found wormy fruit in only one small area at the time of the second picking on July 8, when about one-third of the crop had been harvested. Examination of 5 to 10 peaches per tree found on the ground under 10 trees revealed only 1 wormy peach. The Maygold and Red Cap varieties were also virtually free of worms. In contrast, Hiley peaches in a neighbor's orchard separated by a wooded area were ruined by the plum curculio. The owner of the

first orchard thinks enough of the aldrin soil treatment to use it in his young peach orchards as they reach bearing age.

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# Parathion and EPN Residue Studies on Concord Grapes[1]

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ABSTRACT

Loss of parathion and EPN deposits from the fruit of the Concord grape was determined over a period of 3 weeks following the final application of three-spray programs. Deposits of EPN persisted somewhat longer than those of parathion. The presence of hydrated lime in the spray mixture caused no appreciable increase in rate of loss. A complication was encountered in making determinations on maturing Concord grapes by the Averell & Norris (1948, Analyt. Chem., vol. 20, p. 753) method. This evidence suggests that a color interference in the fruiti s caused by the removal of the methyl anthranilate by the benzene used as a solvent to remove the insecticide deposit. This compound, to a large extent, can be removed by washing the benzene strip several times with 10% hydrochloric acid.

A schedule of three sprays of DDT, if used with a sticking agent, to control the grape berry moth, *Paralobesia viteana* (Clem.), may leave residues in excess of the legal tolerance. These residues are of no consequence when the fruit is to be processed into juice. Quite a different problem is presented, however, when the grapes are to be sold as fresh fruit or are to be used to make preserves. To avoid the possibilities of excessive residues, consideration has been given to the use of two DDT sprays followed by one spray of an organic phosphorous insecticide. Of these latter, parathion and EPN have proved to be very effective when thus used (Taschenberg 1950). These chemicals were also found to be as effective as DDT in a full- or three-application schedule. With the insecticidal performance of parathion and EPN established, attention was directed to a determination of rate of dissipation of spray deposits of these materials from fruits of the Concord variety. Since hydrated lime is generally included with copper in sprays used on this variety, the studies included lots where these materials were present and where they were absent. The results of these investigations are given herein, with observations on the difficulty in determining, by the Averell & Norris (1948) method, the parathion and EPN residues on the maturing fruit.

Considerable study has been made of the persistence of parathion and EPN deposits on fruit and foliage. All reports agree that deposits of both insecticides are relatively short lived. Hamilton (1948) found a 75% loss in parathion deposits on pear fruits 7 days after treatment. King & Hutson (1948, 1949) found that on apples the decrease in parathion deposit was from 3.1 to 0.15 p.p.m. within 3 weeks. Other workers (Snapp 1949, Decker *et al.* 1950, Westlake & Fahey 1950, Walker 1950, Barnes *et al.* 1950) have presented similar data on apples, peaches, and pears. Fahey *et al.* (1952) found EPN more persistent than parathion. They learned further that in the absence of rainfall and under uniform temperatures, the decrease in parathion deposit was a function of the log of time elapsed. Brunson & Koblitsky (1952) recorded these chemicals lost at a slower rate from peach fruit than from foliage. Braid & Dustan (1955) determined the rate of loss of parathion deposit on immature peach fruits. For a spray deposit laid down by 2 pounds of 15% parathion in 100 gallons (imperial) of water the rate of loss was 42 micrograms per 100 cm.$^2$ per day for 12 to 14 days, after which time loss leveled off to a lower value.

METHODS.—The application of spray treatments for this study was made with a hooded boom equipped with 14 nozzles. A detailed description of this equipment is given by Taschenberg (1948). The amount of spray applied was from 225 to 250 gallons per acre, the higher gallonage being used in the final treatment. These amounts are similar to those suggested for commercial spraying of vineyards at the time of this study. The plots were 1 row wide and consisted of 65 vines per row.

The procedure followed in taking samples for residue analyses was similar to that described in an earlier paper (Taschenberg & Avens 1960). Essentially the lots were made up of either 200 or 300 berries taken from 20 vines. In any one test the number of berries taken per sample was constant.

Parathion and EPN deposits were stripped from the samples within 2 hours after collection, by gently rotating the berries in benzene for about 25 minutes.

METHOD OF ANALYSIS.—The method of analysis employed to determine the parathion and EPN deposits was that of Averell & Norris (1948). In one step in this procedure the nitro group of the parathion molecule is quantitatively reduced to an amino group. Then it is diazotized and coupled with N-1-naphthylethylenediamine-dihydrochloride to give an intense magenta color. Aniline and some aniline analogs are known to produce a similar color by this procedure.

INTERFERENCE.—An analog of particular interest in this connection is methyl anthranilate, because it is found in grapes (Power & Chestnut 1923) and in citrus (Small 1943). The amount of this ester increases as the fruit reaches maturity and the content is known to be different in various varieties of grapes (Power & Chestnut, 1923,

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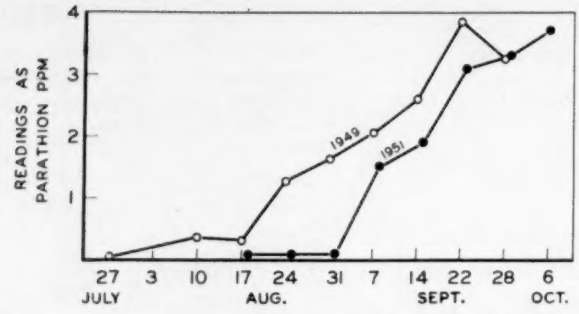


FIG. 1.—Analysis of untreated Concord grapes by the Averell and Norris method showing the degree of methyl anthranilate interference as fruit matures.

Robinson *et al.* 1949). According to the report of Power and Chestnut the methyl anthranilate content of Concord grapes is much higher than in most grape varieties and the accumulation of the ester in fruit of this variety as it reaches maturity is shown in the 1949 publication of Robinson *et al.* Both groups of workers conducted their studies on juice rather than on whole fruit. Evidence of interference in the estimation of parathion residues on the surface of Concord grapes is presented by data obtained in 1949 (Taschenberg 1950). The initial blank value on untreated fruit was found to be zero 36 days prior to harvest. On subsequent samples taken 1 week and 3 weeks later, positive values of 0.32 p.p.m. and 1.82 p.p.m., respectively, were recorded. Presented here are the data from further investigation on the interferences encountered in determining surface residues of parathion and EPN on Concord grapes and several less common varieties.

For two seasons, 1949 and 1951, samples of Concord berries collected from unsprayed vines were subjected to the various steps of the procedure followed in making an analysis for parathion residues. The sampling extended over a period from early August to late September. It is in the latter part of this period that this variety usually reaches maturity. Each sample of berries was stripped for 25 minutes with benzene. The results of the analysis on unsprayed berries is presented graphically in figure 1. As the fruit reaches maturity the amount of interference increases, the spectrophotometer readings calculated as parathion in p.p.m. being zero on the initial samples and reaching 3.6 p.p.m. or more on the later samples. The curves in figure 1 are similar to the curve for methyl anthranilate development in Concord grapes presented by Robinson *et al.* (1949). Samples of untreated berries from several varieties, namely, Van Buren, Fredonia, and Westfield were analyzed by the same method as the samples of Concord berries. According to the data in table 1 the amount of interference differs with the variety. The greater interference is associated with the higher content of methyl anthranilate of the fruit. Both Van Buren and Fredonia were found to have a low methyl anthranilate content when mature (Robinson *et al.* 1949).

REMOVAL OF INTERFERENCE.—Blinn & Gunther (1950) studied the effect of methyl anthranilate in the estimation of parathion by the Averell and Norris method. In their work methyl anthranilate hydrochloride was subjected to the same analytical procedure as used for parathion. Besides showing that methyl anthranilate would interfere in the determination of parathion, they also reported that repeated washings with 10% hydrochloric acid solution removed much of the interference from a benzene solution of this ester. This procedure was tried on benzene strips from samples of untreated grapes. In table 2 are indicated the results with one to six washings of the "strip" with 10% hydrochloric acid. A single washing removed about 76% of the interference and a second washing an additional 10%. Subsequent washings failed to decrease the interference further. From the data in table 2 it is shown that the commercial benzene used to strip fruit gave no interference. Edwards (1949) pointed out the likelihood of interference produced by some grades of this solvent when small amounts of parathion are being determined.

With the more mature samples of Concord grapes untreated fruit may give a value of as much as 4.2 p.p.m. of parathion equivalent. Thus in 100 ml. of the benzene strip there would be 232 micrograms of methyl anthranilate, about three times the quantity of the ester used experimentally by Blinn & Gunther (1950).

Other benzene strips from Concord grapes treated with EPN were washed four times with 10% hydrochloric acid and the removal of interference amounted to about 87%. These data are given in table 3. This evidence sup-

**Table 1.—Methyl anthranilate interference in the analysis of parathion residues on four untreated varieties of grapes.**

| VARIETY | PHOTOMETER READINGS CALCULATED AS PARATHION (P.P.M.) | | | |
|---|---|---|---|---|
| | 1952 | | | |
| | *9/8* | *9/22* | *9/29* | *10/6* |
| Concord | 1.5 | 3.1 | 3.3 | 3.7 |
| Van Buren | 2.0 | 2.7 | — | — |
| Fredonia | .05 | .04 | — | — |
| | 1953 | | | |
| | *9/8* | *9/22* | *10/5* | *9/19* |
| Van Buren | 0.11 | 1.35 | 2.0 | — |
| Fredonia | 0.4 | 0.07 | 0.00 | 0.02 |
| Westfield | 0.08 | 0.11 | 0.02 | 0.04 |

**Table 2.—Influence of washings with 10% hydrochloric acid on the removal of methyl anthranilate from strippings of untreated Concord Grapes.**

| SUBSTANCE[a] ANALYZED | ALIQUOT | NO. OF EXTRACTIONS WITH 10% HCl | INTERFERENCE AS PARATHION (P.P.M.) |
|---|---|---|---|
| Benzene strip | 10/500 | — | 2.27 |
| Benzene strip | 10/500 | 1 | 0.55 |
| Benzene strip | 10/500 | 2 | 0.32 |
| Benzene strip | 10/500 | 3 | 0.32 |
| Benzene strip | 10/500 | 4 | 0.38 |
| Benzene strip | 10/500 | 5 | 0.22 |
| Benzene strip | 10/500 | 6 | 0.32 |
| Commercial benzene | 10 ml. | — | 0.00 |
| Commercial benzene | 25 ml. | — | 0.00 |

[a] Strip from sample of untreated berries collected 10/7/54, weight of sample 954 gms.

**Table 3.—Residues of EPN before and after washing benzene strips from Concord grapes with dilute hydrochloric acid.**

| Treatment[a] | Initial Analyses | | Four Extractions with 10% HCl | |
|---|---|---|---|---|
| | Total (p.p.m.) | Net (p.p.m.) | Total (p.p.m.) | Net (p.p.m.) |
| *Sample collected September 8*[b] | | | | |
| EPN, 1 lb. | 4.10 | 1.10 | 0.80 | 0.43 |
| Untreated | 3.00 | — | 0.37 | — |
| *Samples collected September 15*[b] | | | | |
| EPN, 1 lb. | 4.40 | 0.50 | 0.80 | 0.29 |
| Untreated | 3.90 | — | 0.51 | — |

[a] Final treatment applied 8/11; previous treatments, 6/25 and 7/5.
[b] Samples consisted of 300 berries.

ports the findings of Blinn & Gunther (1950). EPN residue determinations made on samples over a 5-week period are presented graphically in figure 2. The solid-line curve represents the results of analyses on treated berries. The broken line represents the values obtained by deducting the spectrophotometer readings as EPN on untreated fruit from values recorded for treated fruit. It appears doubtful that the usual practice of deducting the values on the untreated from values on treated samples provides an accurate estimation of the amount of residue, especially when there is a sufficiently large amount of interference to give a high value on the blank. Such a procedure is also questionable because of the variation in methyl anthranilate content in fruit from different plots and even between vines in the same plot. By washing the benzene strip four times with dilute hydrochloric acid, residues were recorded of the order that might be expected. The marked increase in interference which appeared to develop within 1 week warrants some explanation. In collection of samples up to and including those picked on Septebmer 1 there was this restriction: only berries showing little or no color were picked. Thereafter

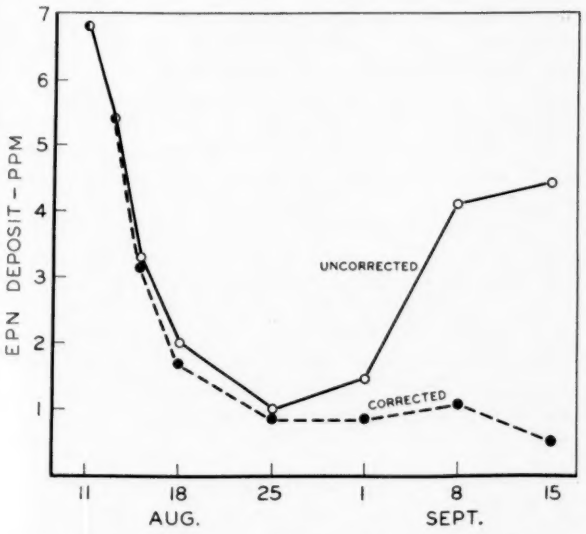


FIG. 2.—Showing disappearance of EPN deposits from Concord grapes with a correction for methyl anthranilate interference.

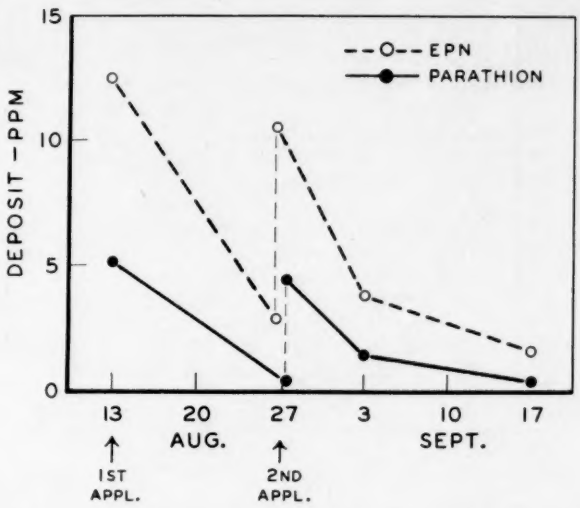


FIG. 3. Showing levels of parathion and EPN deposits following first treatment and before and after the second application, 1951. Note: Parathion (15% w.p.) 2 lb./100 gal. EPN (27% w.p.) 2 lb./100 gal.

no effort was made to follow the restriction because of the difficulty of separating berries according to degree of coloration.

RESULTS AND DISCUSSION.—Only a limited amount of information is available on parathion residues on grapes. No reference to EPN residues on this crop was found in the literature. In 1951, residues were determined on fruit treated with sprays in which 15% parathion or 27% EPN wettable powder formulations were used at rates of 2 pounds in 100 gallons of water. All sprays contained a spreader sticker but no lime. Only two applications were made, with the initial treatments applied August 13 and repeated August 27. The deposit levels of these insecticides from August 13 to September 17 are presented graphically in figure 3. Two weeks after the August 13 applications there was a loss of 94.1% and 77.6%, respectively, of the original deposits of parathion and EPN. With the August 27 treatment the deposits were restored to about the same level that prevailed on August 13. Within 7 days after the second spray 94.1% of the August 27 level of parathion was lost but only 64.2% of the EPN deposit. These findings for parathion are in agreement with those published earlier by Taschenberg (1950). Allowing for the fact that nearly twice as much actual EPN as parathion occurred in spray deposits compared, it is evident that the rate of loss is somewhat slower with EPN.

The studies were continued in 1952 where both insecticides were applied at two concentrations in schedules of three sprays. Both materials were also used alone and in combination with fungicides. In the first treatment, applied immediately after bloom, ferbam was added at the rate of 1.5 lb. per 100 gallons of water. In the second, applied 10 days later, and in the third, applied in August, the spray mixture included 2 pounds of a low-soluble copper fungicide and 4 pounds of hydrated lime. To the above spray mixtures a miscible oil was added to each 100 gallons of spray at the following rates: 8 ounces in

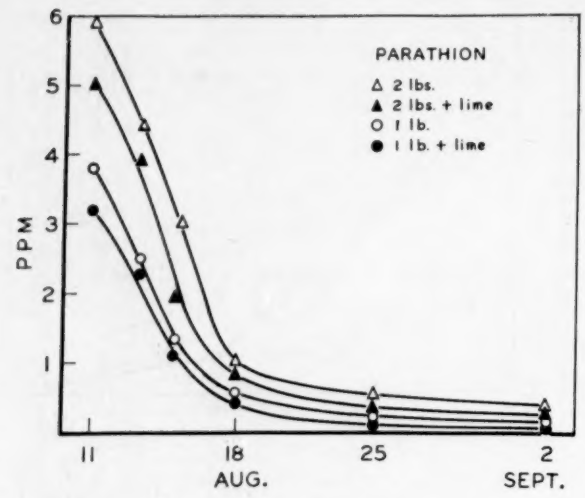


FIG. 4. Showing disappearance of parathion deposits in presence and absence of lime, 1952.

the first treatment and 32 ounces in the second and third treatments. Samples were taken for residue determination in these plots at 2- to 3-day intervals during the first week following the cessation of spraying and thereafter at 7-day intervals.

The parathion levels for the various treatments are shown in figure 4. Similar data on EPN are given in table 4. It appears that deposits of both insecticides are not materially decreased by the presence of hydrated lime in the spray mixture. The small differences found may be partially explained by the nature of coverage laid down by the different spray mixtures. The deposit from the combination of insecticides, fungicide, and the miscible oil tends to be smooth and uniform. Where insecticides are used with only a small amount of miscible oil (8 ounces per 100 gallons) the deposit is usually blotchy. Within the first week following the last application there is a pronounced reduction in amounts of deposits of both parathion and EPN—a slightly greater decrease occurring with parathion. Thereafter, the loss rate tends to level off. At the end of 2 weeks parathion residues were below 1 p.p.m. for sprays containing 2 pounds of this insecticide per 100 gallons. For EPN used at approximately the same actual toxicant rate it was approximately 3 weeks before the residue reached the level of 1 p.p.m. These results were similar to those reported on apples and peaches by Fahey *et al.* (1952). Since the rate of increase of berry weight was slight for the period in which samples were collected, it appears that weathering accounted for most of the loss of insecticide. Data on weight increases for the fruit of Concord grapes between time of last spray and harvest are given in another paper by Taschenberg & Avens (1960).

**Table 4.—Disappearance rate of EPN from spray deposits of the insecticide used alone and with hydrated lime. 1952.**

| Date of Sampling[c,d] | EPN Alone[a] | | | | EPN and Lime[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 lb. Deposit | | 2 lb. Deposit | | 1 lb. Deposit | | 2 lb. Deposit | |
| | P.p.m. | Per Cent Original | P.p.m. | Per Cent Original | P.p.m. | Per Cent Original | P.p.m. | Per Cent Original |
| 8/11 | 6.8 | 100 | 12.0 | 100 | 4.9 | 100 | 10.0 | 100 |
| 8/13 | 5.4 | 79 | 7.6 | 63 | 3.7 | 75 | 6.3 | 63 |
| 8/15 | 3.2 | 47 | 6.2 | 52 | 3.1 | 63 | 4.5 | 45 |
| 8/18 | 1.7 | 25 | 4.5 | 37 | 2.2 | 45 | 2.9 | 29 |
| 8/25 | 0.9 | 14 | 2.1 | 17 | 1.1 | 22 | 1.7 | 17 |
| 9/1 | 0.8 | 12 | 1.6 | 13 | 0.6 | 12 | 1.1 | 11 |

[a] Emulsible oil, 8 ounces.
[b] Spray mixture contained lime, 4 lb; low soluble copper, 2 lb; and emulsible oil 32 oz. per 100 gal.
[c] Final application 8/11; previous applications 6/25 and 7/5. Rainfall accumulated inches 8/11-0.0, 8/13-0.26, 8/15-0.26, 8/18-1.47, 8/25-3.64, 9/1-3.96.
[d] Samples contained 300 berries. Values in p.p.m. on untreated: 8/11-0.0, 8/13-0.0, 8/15-0.13, 8/18-0.3, 8/25-.12, 9/1-0.6, deducted from values in this table.

**Table 5.—Decreases in parathion deposits on berries from clusters selected according to amount of exposure, 1952.**

| Date Sampled[b] | Parathion Deposit[a] | |
|---|---|---|
| | P.p.m. | Per Cent Original |
| | *Regular series*[c] | |
| 8/26 | 4.1 | 100 |
| 8/30 | 1.9 | 46 |
| 9/1 | 1.2 | 29 |
| | *Protected series* | |
| 8/26 | 3.3 | 100 |
| 8/30 | 1.8 | 55 |
| 9/1 | 0.9 | 27 |
| | *Unprotected series* | |
| 8/26 | 4.6 | 100 |
| 8/30 | 1.5 | 33 |
| 9/1 | 0.8 | 17 |

[a] Parathion, 15% w.p., 1.75 lb./100 gal. and DuPont spreader-sticker 4 oz. All samples 200 berries.
[b] Maximum temperature, F° at site of berries reading at 2-day intervals.
Protected: 8/27-8/28, 91; 8/29-8/30, 88; 8/31-9/1, 92.
Unprotected: 8/27-8/28, 104; 8/29-8/30, 99; 8/31-9/1, 100.
Rainfall, 9/1-0.32 inches.
[c] Selection of clusters for sampling according to procedure discussed under "methods."

The influence of the position of clusters on the vines on spray deposit and weathering was also considered in the studies reported here. The samples taken fell into three categories: (1) a regular series, wherein both the more exposed and well protected clusters were avoided in sampling; (2) an unprotected series, which included exposed clusters only; and (3) a protected series, in which the sample was taken from the least exposed clusters. The data obtained in these samplings are given in table 5. As might be expected the lightest initial deposit was found on the protected clusters. However, with respect to percentage, the rate of loss in this series was lower than for the other lots for the first 4 days. The greatest decrease over this period was found on berries from exposed clusters, where it was 83%. This unusually heavy loss was perhaps influenced by high temperatures, which reached about 100° F. for each of the 4 days during the period. Greater persistence of deposit on the more protected clusters undoubtedly accounts for some of the effectiveness of this relatively short-lived insecticide against the grape berry moth.

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# Relationships Between Structure and Insecticidal Activity of Some Organotin Compounds[1]

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ABSTRACT

The toxicities of 42 organotin compounds applied topically to house flies (*Musca domestica* L.) were examined. The $LD_{50}$ values were determined on a molar basis in order to compensate for the large variations in molecular weight due to the diversity of organic radicals.

Maximum toxicity was obtained with the trisubstituted organotin compounds. Unsymmetrical trivinyltins were of the same magnitude of toxicity as their saturated and symmetrical counterparts. The anionic species in the trisubstituted organotins can be variable and probably contribute little to the toxicities manifested by these compounds. The nature of the aliphatic substituents also is not critical to toxicity.

Di- and tetrasubstituted organotins are of similar toxicity whereas the monosubstituted organotins are least toxic. In the disubstituted organotins the nature of the anion appears to affect the toxicity of the compounds.

Although the toxicity of the heavy metals (Pb, Hg, Sb) to animals has been investigated widely, the toxicity of tin has received little consideration. Whereas inorganic tin has been reported to have negligible toxicity to animals, tin administered in the organic state has been demonstrated to be poisonous. White (1886) showed that triethyltin acetate was highly toxic to mammals in contrast to inorganic tin salts. Collier (1929) compared the toxicities of different organotin compounds to mice and found that triphenyltin bromide was more toxic than several tetraorganotins.

Extensive investigations on the fungitoxicity of organotin compounds were made by van der Kerk & Luijten (1954). They tested a series of ethyltin chlorides and found that the triethyl derivative was the most fungitoxic. The monoethyl derivative was the least toxic while the di- and tetraethyl compounds were of intermediate toxicity. Both stannous and stannic chloride were nontoxic. Van der Kerk & Luijten (1954) and more recently Rosenberg *et al.* (1959) demonstrated that the nature of the anion had little effect on the fungitoxicity of a series of triethyltin derivatives. Also, in a series of trialkyltin acetates, van der Kerk and Luijten showed that toxicity was little affected by the nature of the alkyl groups having up to six carbons through tri-*n*-hexyltin. In a more recent investigation van der Kerk & Luijten (1956) found that the fungitoxicity of several unsymmetrical trialkyl-

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tin compounds was not associated with the nature of the alkyl groups but rather with the total number of carbon atoms present. Maximum fungitoxicity was present in those compounds containing 9 to 12 carbon atoms exclusive of the anionic species.

Stoner *et al.* (1955) examined the toxicity and pharmacology of a number of organotins using rats, rabbits and domestic fowl. They reported that the triethyltin compounds were the most toxic; the di- and tetraethyl derivatives less toxic and the monoethyl compound the least toxic. They also observed that the symptoms of diethyltin and triethyltin poisoning were different, indicating different modes of action for these compounds. On the other hand, the symptoms of tetraethyltin poisoning, although delayed in onset, were identical with those of the triethyltin compound, leading these authors to suggest the possible *in vivo* conversion of tetraethyltin to a triethyltin derivative. A biochemical explanation for the different symptoms of poisoning of the di- and triethyltin compounds observed by Stoner *et al.* was furnished by Aldrich & Cremer (1955) who demonstrated that triethyltin sulphate inhibited phosphorylative processes associated with oxidation and diethyltin dichloride inhibited *l*-keto acid oxidases.

Stoner *et al.* (1955) also demonstrated that high doses of triethyltin sulphate caused immediate death when intravenously administered to rats. The rapid action of this compound was not associated with cholinesterase inhibition. Chronic doses of triethyltin caused extensive cellular damage to the central nervous system but had no effect on other organs. More recent investigations by Barnes & Stoner (1958) have confirmed the central nervous activity of the triethyl derivatives and demonstrated that the diethyl compounds damage the biliary tract.

Blum & Bower (1957) reported that triethyltin hydroxide was capable of causing rapid paralysis of house flies (*Musca domestica* L.) after topical application. Esters of this compound did not inhibit cholinesterase but did block conduction in the isolated central nerve cord of the American cockroach (*Periplaneta americana* (L.)).

Although trialkyltins appear to be quite toxic to mammals, the possibility of obtaining triorgano derivatives of tin with low mammalian toxicity has been realized. The acute oral $LD_{50}$ of bis(tri-*n*-isobutyltin) oxide in corn oil to male albino rats is 148 mg./kg. and in aqueous suspension it is 194 mg./kg. (Elsea & Paynter 1958). No evidence of sensitization to this compound was observed.

It is the purpose of this paper to compare the insecticidal activity of 42 organotin compounds from the standpoint of establishing relationships between structure and toxicity.

EXPERIMENTAL.—House flies used in this investigation were the C.S.M.A. (Chemical Specialties Manufacturers Association) susceptible strain. The larvae were reared on moist Purina dog pellets in a room held at 32° C. ± 1° and 50% ± 5% relative humidity. Adults were held at the same conditions and fed a water suspension of powdered skimmed milk.

Twenty adult female house flies, 3 to 4 days old, were lightly anaesthetized with carbon dioxide and treated topically with 1 μl. of an acetone solution of the organotin compound. Two compounds, tetra-*n*-lauryltin and di-*n*-octyltin dilaurate were insoluble in acetone, and therefore were dissolved in an emulsifier, Emulphor EL-719.[3] The solution was administered from a syringe, the plunger of which was driven with a micrometer. Four samples of 20 flies each from each of two populations were run on every compound. After treatment, the flies were held in half-pint Mason jars at the previously stated constant temperature and humidity.

The maximum amount of compound applied to a fly was 12 μg. Compounds whose $LD_{50}$ was above this dose are listed in table 1 as 12 μg. with the mortality obtained placed in parentheses. The per cent mortality obtained with acetone alone was 5 ± 2% and with Emulphor EL-719 7 ± 3%.

RESULTS AND DISCUSSION.—The results of this study are presented in table 1. The compounds discussed are referenced by numbers in the table.

The $LD_{50}$'s of the various compounds are expressed in moles. The molar value compensates for differences in molecular weights of the compounds and permits a more exact measurement of the inherent toxicity of the molecule. In this way, direct comparisons may be made between compounds which contain dissimilar anions. The molar $LD_{50}$ figure therefore truly reflects the toxicity of a compound, since this value is a measurement of the number of molecules involved in the response being measured.

*Stannous Carboxylates, Monoaryl and Monoalkyl Tin Derivatives.*—Stannic chloride (1) and the non-alkylated stannous carboxylates (2 and 3) were almost nontoxic at the 12-μg. dose level, as were the monoarylated and monoalkylated tin compounds, phenyltin trichloride (4) and *n*-butyltin-tri(2-ethylhexanoate)(5). These results are in agreement with the findings of Stoner *et al.* (1955) on mammals and van der Kerk & Luijten (1954) on fungi, that monoalkyltins are the least toxic of the alkylated tin derivatives.

*Dialkyl and Diaryl Tin Derivatives.*—In comparing compounds 4 with 10 and 5 with 12, an increase in toxicity is associated with replacement of one of the anionic groups with a butyl (12) or with a phenyl (10) radical. In the dialkyl tin dichlorides, maximum toxicity occurs in the dimethylated compound (6). Barnes & Stoner (1958) reported that the higher dialkyl tin dichlorides were less toxic to rats than their lower homologs. The nature of the anionic species in the dialkyltin compounds seems to influence the toxicity of the molecule, since the toxicities of the diethyl (7) and dioctyl (9) tin dichlorides are significantly greater than their respective ethyl (11) and octyl (13) carboxylate counterparts. This fact is also evident when dibutyltin dichloride (8) is compared with di-*n*-butyl-di(2-ethylhexanoate)(12), although in this case the carboxylate is more toxic than the halide.

*Trialkyl and Triaryl Tin Derivatives.*—The triorganotins (14 to 32) were the most toxic compounds studied. This is especially evident in the series of arylated tin derivatives (4, 10, 27 and 43) in which the triarylated species (27) is the only significantly toxic compound.

In the trisubstituted tin derivatives, the effect of the anion on toxicity is less than that found in the diorganotin series. Among the triethyltin derivatives (15 to 19), the influence of OH, $O_2CCH_3$, $SCH_2O_2C_2H_5$, $SC_6H_5$ and

[3] Polyoxyethylated Vegoil from Antara Chemicals, New York, N. Y.

**Table 1.—Twenty-four hour $LD_{50}$ values of organotin compounds applied topically to susceptible house flies.**

| COMPOUND | FORMULA | $LD_{50}$ (MOLES$\times10^{-10}$/FLY)[a] |
|---|---|---|
| 1. Stannic chloride | $SnCl_4$ | >460 (20)[b] |
| *Stannous carboxylates* | | |
| 2. Stannous octanoate | $Sn[O_2C(CH_2)_6CH_3]_2$ | >300 (15) |
| 3. Stannous oleate | $Sn[O_2C(CH_2)_7CH=CH(CH_2)_7CH_3]_2$ | >180 (10) |
| *Monoaryl and monoalkyl tin derivatives* | | |
| 4. Phenyltin trichloride[e] | $(C_6H_5)SnCl_3$ | >390 (15) |
| 5. *n*-butyltin-tri(2-ethylhexanoate)[c] | $n\text{-}C_4H_9Sn[O_2CCH(C_2H_5)(CH_2)_3CH_3]_3$ | >160 (30) |
| *Dialkyl and diaryl tin derivatives* | | |
| 6. Dimethyltin dichloride[e] | $(CH_3)_2SnCl_2$ | 120 |
| 7. Diethyltin dichloride[d] | $(C_2H_5)_2SnCl_2$ | 410 |
| 8. Di-*n*-butyltin dichloride[e] | $(n\text{-}C_4H_9)_2SnCl_2$ | 340 |
| 9. Di-*n*-octyltin dichloride[d] | $(n\text{-}C_8H_{17})_2SnCl_2$ | 250 |
| 10. Diphenyltin dichloride[e] | $(C_6H_5)_2SnCl_2$ | 290 |
| 11. Diethyltin diacetate[e] | $(C_2H_5)_2Sn(O_2CCH_3)_2$ | >410 (10) |
| 12. Di-*n*-butyltin-di(2-ethylhexanoate)[c] | $(n\text{-}C_4H_9)_2Sn[O_2CCH(C_2H_5)(CH_2)_3CH_3]_2$ | 130 |
| 13. Di-*n*-octyltin dilaurate[e] | $(n\text{-}C_8H_{17})_2Sn[O_2C(CH_2)_{10}CH_3]_2$ | >220 (45) |
| *Trialkyl and triaryl tin derivatives* | | |
| 14. Trimethyltin bromide[e] | $(CH_3)_3SnBr$ | 4.5 |
| 15. Triethyltin hydroxide | $(C_2H_5)_3SnOH$ | 11.0 |
| 16. Triethyltin acetate | $(C_2H_5)_3SnO_2CCH_3$ | 11.0 |
| 17. Triethyltin ethyl mercapto acetate[e] | $(C_2H_5)_3SnSCH_2CO_2C_2H_5$ | 4.2 |
| 18. Triethyltin phenyl mercaptide[f] | $(C_2H_5)_3SnSC_6H_5$ | 5.0 |
| 19. Triethyltin benzyl mercaptide[f] | $(C_2H_5)_3SnSCH_2C_6H_5$ | 5.6 |
| 20. Trivinyltin iodide[e] | $(CH_2=CH)_3SnI$ | 15.0 |
| 21. Triisopropyltin acetate | $(i\text{-}C_3H_7)_3SnO_2CCH_3$ | 24.0 |
| 22. Triisopropyltin undecylenate[e] | $(i\text{-}C_3H_7)_3SnO_2C(CH_2)_9CH_3$ | 16.0 |
| 23. Tri-*n*-butyltin chloride[e] | $(n\text{-}C_4H_9)_3SnCl$ | 4.6 |
| 24. Tri-*n*-butyltin octanoate | $(n\text{-}C_4H_9)_3SnO_2C(CH_2)_6CH_3$ | 5.6 |
| 25. Tri-*n*-butyltin-dibutoxy-dithio-phosphate[e] | $(n\text{-}C_4H_9)_3S\text{-}P(=S)(O\text{-}n\text{-}C_4H_9)_2$ | 9.3 |
| 26. Tris-(tri-*n*-butyltin) perthiophosphate[e] | $[(n\text{-}C_4H_9)_3SnS]_3P=S$ | 9.0 |
| 27. Triphenyltin chloride[e] | $(C_6H_5)_3SnCl$ | 3.9 |
| 28. Dimethyl vinyltin chloride[e] | $(CH_3)_2(CH_2=CH)SnCl$ | 7.1 |
| 29. Dimethyl vinyltin-$\alpha$-bromopropionate[e] | $(CH_3)_2(CH_2=CH)SnO_2CCHBrCH_3$ | 7.4 |
| 30. Divinyl-*n*-butyltin chloride[e] | $(CH_2=CH)_2(n\text{-}C_4H_9)SnCl$ | 12.0 |
| 31. Vinyl di-*n*-dibutyltin chloride[e] | $(CH_2=CH)(n\text{-}C_4H_9)_2SnCl$ | 12.0 |
| 32. Divinyl phenyltin fluoride[e] | $(CH_2=CH)_2(C_6H_5)SnF$ | >500 (10) |
| *Alkyl tin oxide and alkyl distannane* | | |
| 33. Bis(tri-*n*-butyltin)oxide[e] | $(n\text{-}C_4H_9)_3Sn\text{-}O\text{-}Sn(n\text{-}C_4H_9)_3$ | 11.0 |
| 34. Hexa-*n*-butyl distannane[e] | $(n\text{-}C_4H_9)_3Sn\text{-}Sn(n\text{-}C_4H_9)_3$ | >210 ( 5) |
| *Tetraalkyl and tetraaryl stannanes* | | |
| 35. Tetramethyltin[e] | $(CH_3)_4Sn$ | >560 ( 0) |
| 36. Dimethyl divinyltin[e] | $(CH_3)_2(CH_2=CH)_2Sn$ | 530 |
| 37. Tetravinyltin[e] | $(CH_2=CH)_4Sn$ | >530 ( 0) |
| 38. Tetraethyltin[e] | $(C_2H_5)_4Sn$ | 330 |
| 39. Tetraisopropyltin[e] | $(i\text{-}C_3H_7)_4Sn$ | >415 (40) |
| 40. Tetra-*n*-butyltin[e] | $(n\text{-}C_4H_9)_4Sn$ | 290 |
| 41. Tetra-*n*-octyltin[e] | $(n\text{-}C_8H_{17})_4Sn$ | 196 |
| 42. Tetra-*n*-lauryltin[e] | $(n\text{-}C_{12}H_{25})_4Sn$ | >150 (25) |
| 43. Tetraphenyltin[e,g] | $(C_6H_5)_4Sn$ | >290 (30) |

[a] Molar values were calculated also for compounds whose $LD_{50}$ values exceed 12 $\mu$g. in order to indicate the maximum number of applied moles not capable of killing 50% of the population.

[b] Figures in parentheses in this column indicate per cent mortality when the flies were treated with 12 micrograms of the test material.

[c] Obtained from Dr. E. L. Weinberg, Metal & Thermit Corp., Rahway, N. J.

[d] Obtained from Dr. Frank A. Bower, E. I. DuPont Co., Wilmington, Del.

[e] Obtained from Dr. Dietmar Seyferth, Harvard University, Cambridge, Mass.

[f] Obtained from Dr. George S. Sasen, Drexel Institute of Technology, Philadelphia, Pa.

[g] Insoluble in everything except hot benzene. Therefore, this compound was evaluated as a residue of 50 mg./sq. ft. The 12 $\mu$g. $LD_{50}$ is an estimation of the low toxicity of this compound expressed for comparative purposes.

$SCH_2C_6H_5$ on toxicity appears to be negligible, although the mercaptides (17, 18, 19) appear to be slightly more toxic than either the hydroxide and acetate; or perhaps the sulfur-containing anions contribute to the toxicities of the molecules. On the other hand, Blum & Bower (1957) reported that increasing the size of the carboxyester moiety of triethyltin derivatives reduced the toxicity of the compound but none of the compounds contained sulfur.

The relatively uniform toxicity of the trisubstituted tin derivatives further illustrates the nonspecific effect of both the cationic and anionic groups on insecticidal activity. It seems reasonable to assume that toxicity is independent of the numerous anionic species and is correlated with the presence of three organic groups in the tin atom's tetrahedral structure, as van der Kerk & Luijten (1954) demonstrated with fungi.

The unsymmetrical vinyltins (28 to 32), with the exception of divinyl phenyltin fluoride (32), are typical toxic triorganotins. The toxicity of these compounds compares favorably with the symmetrical triorganotins (14 through 27) further demonstrating that the nature of the

polyorganic groups is not critical to toxicity of the triorganotins. Interestingly, although aliphatic dehydrogenation often increases the toxicity of pharmacologically active compounds, no demonstrable toxicity increase was evident when vinyl was substituted for saturated radicals in this series (compare 20 with 16). Divinyl phenyltin fluoride (32) is relatively inactive, a fact which possibly may be correlated with the physical properties of the compound. The introduction of anionic fluorine confers on the compound properties that are more inorganic than organic. This chemical is only slightly soluble in most organic solvents and has a melting point of more than 300° C. (Seyferth 1956), a property which indicates the near inorganic state of the compound. The polarity of the compound probably would allow it to penetrate the insect integument very poorly.

The alkyl distannane (34) and oxide (33) present an interesting structure-toxicity study. The oxide (33) is toxic to house flies, whereas the distannane (34) is not. The oxide is susceptible to hydrolysis and ionization (Weinberg 1956) but the distannane chemically resembles a tetraorganotin and is far less susceptible to hydrolysis than the oxide (Seyferth 1956). Luijten & van der Kerk (1955) have shown that triethyltin and diethyltin exist as ions in aqueous solution. Therefore, it is possible that organotins may exert their toxic action as ions. Many of the mono-, di- and trisubstituted organotin compounds used in this study are capable of aqueous ionization (Weinberg 1956) and probably all of them are to some extent. Although the relationship of ionization to toxicity has not been studied for the compounds evaluated in this paper, the importance of ionization in organotins deserves attention.

The high toxicity of bis(tri-*n*-butyltin) oxide to house flies, and its low mammalian toxicity (Elsea & Paynter 1958) indicate a possible difference between the biochemical fates of this compound in mammals and in insects, and suggest the possibility of finding other selectively toxic organotin compounds.

*Tetraalkyl and Tetraryl Tin Derivatives.*—The tetrasubstituted organotins (35 through 43) do not exhibit much insecticidal activity and approximate the toxicities of the disubstituted organotins (6 through 13). The most toxic member of this series was tetra-*n*-octyltin (41). Toxicity increased regularly with chain length up to eight carbon atoms, except in the case of tetraisopropyltin (39). Replacement of two methyl groups with vinyl species (35, 36) does not affect toxicity, while replacement of the ethyl species of tetraethyltin with vinyl groups destroys toxicity (37, 38). Whereas phenyl compares favorably with various alkyls as a tin substituent in the di- and trisubstituted organotins, the derivative produced by the arylation of tin is far less toxic (43) than the alkyls in the tetrasubstituted series. Since cuticular penetration occurs most rapidly in the case of fat soluble materials and tetraphenyltin is relatively insoluble in most fat solvents, its lack of toxicity may possibly be attributable to poor penetration.

Stoner *et al.* (1955) reported that the onset of toxic symptoms of tetraethyltin on the rat were delayed. These delayed symptoms simulated those of triethyltin, leading these authors to suggest the *in vivo* conversion of tetraethyltin to a triethyltin derivative. Cremer (1958) confirmed the hypothesis of Stoner *et al.* (1955) by demonstrating that tetraethyltin was converted to a triethyl derivative, mainly by the microsomes and soluble matter from liver cells. An examination of the 72-hour mortalities of house flies treated with tetraethyltin and tetra-*n*-butyltin revealed no delayed symptoms of poisoning.

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# Further Studies on Control of the Clover Root Borer in Virginia[1]

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Abstract

The clover root borer, *Hylastinus obscurus* (Marsh.), was effectively controlled in red clover by granulated insecticides. Heptachlor at 0.6 pound, BHC at 1 pound of the gamma isomer and aldrin at 0.7 pound per acre were effective for one season. Heptachlor at 1 pound per acre gave good control for two seasons, and at 2 pounds gave satisfactory control for three seasons.

Woodside & Turner (1956) reported that the clover root borer, *Hylastinus obscurus* (Marsh.), could be controlled effectively in red clover by any one of several insecticides in granulated form. They also found that increases in hay yield at the second or the second and third cuttings followed when this insect was controlled in red clover. A study of this problem in Virginia has been continued in an effort to determine the most satisfactory insecticide for control of the clover root borer and to ascertain the lowest feasible rate of treatment.

Methods and Materials.—BHC, heptachlor, chlordane, and aldrin were tested as granules. Heptachlor and BHC were tested as sprays in 1956, and heptachlor was tested as a spray and as a dust in 1957. Most of the granulated formulations were on attaclay of 30/60 mesh, but the BHC and one formulation of heptachlor were on granulated tobacco stems of 30/60 mesh. The carrier and the toxicant content of each formulation are shown in the footnotes to the tables.

The experiments were conducted in Augusta County, with the exception of the fifth experiment, which was located in Rockbridge County. Each plot contained 0.01 acre, being 10 feet wide and 43.6 feet long. A randomized block arrangement of plots with five replications was used in all experiments except the fifth, where there were four replications. Except for the 1957 treatments in the fifth experiment, all treatments were applied to red clover that had been seeded the previous spring, so that the clover was 6 months to 1 year old when treated. In the fifth experiment the two 1957 treatments were applied to the soil just before the clover was seeded in April, and the insecticide was worked lightly into the soil before the clover seed was sown. The two 1958 treatments in this experiment were applied to the 1-year-old clover in the same manner as in the other experiments.

The granulated insecticides were applied with a small lawn fertilizer spreader. The amount of insecticide delivered was determined by weighing the material placed in the spreader and the amount left after the treatment was completed. The rate of application was not always that which had been planned. The sprays were applied with a compressed air sprayer, and the dusts with a hand duster. The amount of spray material or dust for each plot was weighed separately and the exact amount applied.

In July or August of each year 10 clover plants were dug from each plot, each root and crown was dissected and the numbers of clover root borers of all stages in each plant were determined. The seasons of 1956, 1957, and 1958 were dry, and clover made very poor growth for the second cuttings. For this reason no hay samples were taken to determine yields. Although the survival of plants in selected quadrats in each plot was determined by counts made in June and September each year, the results show so little correlation with the infestation by clover root borer that they are not given.

**Table 1.—Results of experiments for control of the clover root borer, Augusta County, Virginia.**

| Insecticide[a] | Pounds of Toxicant per Acre | Date of Treatment | Clover Root Borers per 10 Plants[b] |
|---|---|---|---|
| | *First Experiment, 1955–56* | | |
| Heptachlor | 1.0 | March 1, 1956 | 0 |
| BHC | 1.0 (g.i.) | March 1, 1956 | 0.2 |
| Heptachlor | 1.0 | October 20, 1955 | 0.2 |
| Chlordane | 2.5 | April 12, 1956 | 0.6 |
| Heptachlor[c] | 1.0 | April 12, 1956 | 0.8 |
| | 1.0 | April 12, 1956 | 1.2 |
| Chlordane | 2.5 | October 20, 1955 | 1.8 |
| | 2.5 | March 1, 1956 | 1.8 |
| BHC | 1.32 (g.i.) | October 20, 1955 | 2.8 |
| BHC, spray | 1.0 (g.i.) | March 1, 1956 | 3.0 |
| Heptachlor, spray | 1.0 | April 12, 1956 | 9.4 |
| Untreated | — | — | 40.2 |
| | *Second Experiment, 1956* | | |
| Heptachlor | 0.9 | April 13 | 0 |
| | 0.7 | April 13 | 0 |
| Aldrin | 0.8 | April 13 | 0 |
| Heptachlor | 0.9 | May 5 | 0.2 |
| | 0.7 | April 25 | 0.2 |
| | 0.9 | April 25 | 0.6 |
| Aldrin | 0.96 | April 13 | 0.8 |
| Heptachlor | 0.7 | May 5 | 2.6 |
| | 0.55 | April 13 | 3.0 |
| Untreated | — | — | 36.4 |
| | *Third Experiment, 1957* | | |
| Aldrin | 0.84 | April 26 | 0.2 |
| | 0.72 | April 12 | 1.2 |
| Heptachlor | 0.31 | April 12 | 1.4 |
| | 0.98 | April 12 | 1.8 |
| | 0.98 | April 26 | 2.0 |
| Aldrin | 0.84 | April 12 | 2.6 |
| Heptachlor | 0.44 | April 12 | 2.8 |
| | 0.44 | April 26 | 2.8 |
| Aldrin | 0.72 | April 26 | 3.4 |
| Heptachlor, spray | 1.0 | April 12 | 4.6 |
| Heptachlor, dust | 1.0 | April 12 | 6.6 |
| Heptachlor | 0.31 | April 26 | 12.6 |
| Untreated | — | — | 76.0 |
| | *Fourth Experiment, 1958* | | |
| Heptachlor | 0.7 | April 10 | 0.2 |
| | 0.6 | April 10 | 0.2 |
| | 0.4 | April 10 | 4.2 |
| Untreated | — | — | 24.2 |

[a] Except where otherwise noted, the heptachlor was a 2.5% formulation on 30/60 mesh attaclay, the aldrin a 30% formulation on 30/30 mesh attaclay, and the chlordane a 5% formulation on 30/60 mesh attaclay. The heptachlor on tobacco was a 5% formulation on 30/60 mesh granulated tobacco, and the BHC was a granulated tobacco formulation of 30/60 mesh carrying 2% of the gamma isomer.

[b] All figures flanked by the same line are not significantly different at the 5% probability level.

[c] Heptachlor, 5%, on granulated tobacco stems of 30/60 mesh.

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Table 2.—Results of a fifth experiment for control of the clover root borer, Steeles Tavern, Virginia. 1957–58.

| Pounds of Toxicant per Acre[a] | Date of Treatment | Clover Root Borers per 10 Plants[b] 1958 | 1959 |
|---|---|---|---|
| 1 | April 1958 | 0 | 1.5 |
| 2 | April 1957 | 0.25 | 4.8 |
| 0.5 | April 1958 | 0.13 | 10.5 |
| 1 | April 1957 | 1.0 | 12.5 |
| None | — | 9.88 | 21.8 |

[a] All treatments consisted of a 2.5% formulation of heptachlor or 30/60 mesh attaclay.

[b] All figures flanked by the same line are not significantly different at the 5% evel of probability.

Results.—The results of these experiments are shown in the accompanying tables. In the first experiment (table 1) insecticides were applied in the fall, late winter, and spring. No significant differences appeared between the results obtained with heptachlor, BHC, and chlordane, at the rates used; also no significant differences owing to time of application were present. The spray treatments were somewhat less effective than the granules, the heptachlor spray being significantly so.

In the second experiment in 1956 (table 1) various rates of treatment with heptachlor and aldrin were tested, with the heptachlor applied on two different dates. No significant differences were found between the results of these treatments. It is somewhat surprising that treatments applied as late as May 5 were effective in reducing sharply the infestation of clover root borer. Heptachlor at 0.55 pound per acre gave satisfactory control.

In the third experiment, conducted in 1957 (table 1), aldrin and heptachlor were applied at two dates in April. There were no significant differences between the results of the treatments, except that heptachlor at 0.31 pound per acre applied on April 26 gave significantly poorer control than most of the other treatments. Heptachlor at 0.44 pound per acre gave good control.

In the fourth experiment, conducted in 1958 (table 1), heptachlor was used at several rather low rates. Although the control at 0.4 pound per acre was not significantly poorer than that at higher rates, this low rate of application cannot be recommended unless further experiments show that it is satisfactory.

In the fifth experiment (table 2) applications of 1 or 2 pounds of heptachlor per acre made in April 1957 gave good control of the clover root borer during the 1958 season. In 1958 there were no significant differences between the results obtained with these dosages and those with dosages of 0.5 and 1 pound per acre applied in April 1958. In 1959 the 2-pound dosage applied in 1957 and the 1-pound dosage applied in 1958 still gave good to fair control. The results of the 1-pound treatment made in 1957 and the 0.5-pound treatment made in 1958 were significantly lower, but these gave a significant reduction in infestation from that in the check plots.

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# Face Fly Control[1]

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### Abstract

Face flies (*Musca autumnalis* DeGeer) became numerous in the northern half of Illinois during the summer of 1959. Large-scale residual treatments of farm premises and animals were not effective in controlling face flies. Repellent treatments with R-326 (di-*n*-propyl isocinchomeronate) or Tabatrex (di-*n*-butyl succinate) gave a practical level of control. Treatments consisting of 1% to 2% dimethoate and/or 0.1 to 0.2% DDVP in a syrup bait applied to the foreheads of cattle at an average dosage of 3 ml. per cow eliminated most of the face flies.

In 1959, for the first time, face flies, *Musca autumnalis* DeGeer, invaded Illinois and became numerous enough in the northern half of the State to cause concern among dairy and beef herdsmen. The infestation became serious in late July and August, when most infested herds averaged 20 to 60 flies per animal, with some as high as 100.

A considerable number of methods of control were investigated to determine their effectiveness and practical application in abating face flies. Residual treatments of outside resting sites on barns, sheds, wooden fences, trees, et cetera, with Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dimethoate® *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethol) phosphorodithioate), malathion, and ronnel proved ineffective. Field observations indicated that most of the face flies were to be found in large numbers on fence posts, wires, and vegetation in and around the pasturing areas of the herd. The population density was greatest near the pasturing animals but was found to extend 200 to 250 yards around the animals. Some of the face flies followed the cattle from the pasture into the barn lot but hardly any entered the barn.

Saturation spraying of animals with DDT, malathion, ronnel, and toxaphene emulsions likewise did not achieve a significant level of control.

Repellent treatments of the animals' heads with Tabatrex (di-*n*-butyl succinate) or R-326 (di-*n*-propyl isocinchomeronate) were moderately successful for about a day and resulted in 70% to 90% reductions of flies on the treated areas. However, when face flies were very abundant, a large number would be found on the brisket, shoulders, and sides of the animals or in other places where salivary and blood deposits were prevalent.

Poison Baits.—Our first successful experiments for

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**Table 1.—Preliminary bait treatments where 2 to 4 ml. of syrup bait were applied to the forehead of cattle in the evening for the control of face flies.**

| Toxicant in Bait | Average Number of Face Flies per Cow | | | Per Cent Reduction |
|---|---|---|---|---|
| | Before | After 16–24 Hrs. | Check Herd | |
| 1.0% Diazinon (WP) | 71.2 | 35.0 | 37.7 | 50.8 |
| 1.0% Diazinon (E) | 58.6 | 30.0 | 30.1 | 48.9 |
| 1.0% dimethoate | 59.0 | 15.2 | 35.0 | 74.4 |
| 0.1% DDVP | 48.8 | 7.5 | 35.5 | 84.6 |
| 1.0% dimethoate | 78.6 | 19.1 | 35.0 | 75.7 |

the control of face flies were effected with poison baits applied to the foreheads of the cattle. Initially baits were prepared with corn syrup as a base, proteose-peptone, urea, and the inorganic salts found in saliva as supplements, and an organophosphate as a toxicant. Through experimentation, the supplements were eliminated without reducing the attractiveness of the baits. The simplest and most effective baits contained 75% corn syrup and 25% water, to which DDVP and/or dimethoate were added. Fly acceptance of the bait was materially influenced by the toxicant as well as the formulation. Baits containing the water-soluble organophosphates DDVP and dimethoate proved much more attractive than those containing the insoluble poisons, such as Diazinon.

Baits were in most cases applied to the animals by means of a 1-inch paint brush. An average of 3 ml. of a bait was left upon the forehead of an animal by a single 6-inch stroke of the brush. In a few of our tests a small hydraulic sprayer was used to apply a solid stream of the bait solution. The cattle did not appreciate having a solid stream of bait squirted on their foreheads.

SCREENING TESTS.—Screening tests were conducted to find the most effective bait formulation (table 1). Emulsifiable and wettable Diazinon, when used in baits, was found to be somewhat repellent to the face flies. DDVP and dimethoate, as shown by data given in tables 1 and 2, were very acceptable and effected excellent kill and consequent reductions of face flies that in some cases closely approached eradication.

Bait applications made in the evening were subject to leaching by high humidities and heavy dew; little of the bait material was left to kill flies on the following day.

Morning treatments (table 2) gave excellent killing action through the day, and the bait became dry enough by evening to withstand the moisture during the night. A count of face flies upon the head of an animal revealed that at any one time during the day approximately 60% of the flies were feeding upon the bait. Flies taking moisture around the nose and eyes of an animal soon moved up to the bait station to satisfy their appetites. These observations account for the near eradication at times effected by the bait technique.

**Table 2.—Preliminary bait treatments where 2 to 4 ml. of syrup bait were applied to the forehead of cattle in the morning for the control of face flies.**

| Toxicant in Bait | Average Number of Face Flies per Cow | | | | Per Cent Reduction |
|---|---|---|---|---|---|
| | Before | After 4–36 Hrs. | Residual Kill | Check | |
| 1.0% dimethoate | 23.9 | 1.9 | 3–4 days | 21.3 | 92.4 |
| 0.1% DDVP | 19.8 | 0.3 | 12–36 hrs. | 24.2 | 98.4 |
| 0.1% DDVP +1.0% Diazinon | 20.3 | 2.0 | 2 days | 26.5 | 90.1 |
| 1.0% dimethoate | 28.8 | 4.8 | 2–3 days | 28.1 | 83.3 |
| 1.0% Diazinon (E) | 24.0 | 12.0 | Rain | 25.3 | 50.0 |
| 0.1% DDVP | 26.7 | 0.6 | 12–36 hrs. | 22.4 | 97.5 |
| 2.0% dimethoate | 24.2 | 2.5 | 3–4 days | 22.7 | 89.6 |

**Table 3.—Per cent reduction of face flies effected by the application to the forehead of 2 to 4 ml. bait per cow.[a]**

| Toxicant in Bait | No. Cows Treated | Average Number of Face Flies Before Treatment | Reduction After Treatment (%) | | | Residual Killing Action (Days) |
|---|---|---|---|---|---|---|
| | | | 1–6 Days | 7–13 Days | 14–21 Days | |
| 0.2% DDVP +1.0% dimethoate | 55 | 18.1 | 89.0 | 96.1 | 98.9 | 3–5 |
| 0.2% DDVP +1.0% dimethoate | 26 | 16.3 | 88.7 | 93.9 | 97.5 | 3–5 |
| 0.2% DDVP +1.0% dimethoate | 29 | 37.5 | 86.9[b] | 90.3 | 99.3 | 2–5 |
| 0.2% DDVP +1.0% dimethoate | 25 | 46.5 | 80.2[c] | 92.7 | 94.8 | 1–5 |

[a] Retreatment made as often as necessary to maintain a lethal deposit of bait.
[b] 0.2% DDVP (Daily treatments) for the first week.
[c] 0.1% DDVP (Daily treatments) for the first week.

Face flies fed upon baits containing DDVP for about 40 seconds before they dropped to the ground. Dimethoate acted more slowly and required about 20 minutes before intoxication ensued. The residual killing action of DDVP was limited largely to the first day, whereas dimethoate exerted its lethal action for 2 to 5 days, depending upon environmental conditions.

After the screening test, continuous treatments were inaugurated to ascertain the period required to reduce the face fly population to an insignificant level. Baits were applied daily for the first week, then at 2- to 5-day intervals, depending upon the weather and the abundance of face flies. The syrup bait for this experiment contained 0.2% DDVP and 1.0% dimethoate. This bait combined speed of action and residual kill of the two organophosphates. Data in table 3 indicate a progressive reduction of face flies over a 3-week period, which was attributed to the attrition of the breeding population.

Factors affecting the efficiency of bait treatments were found to be weather, number of animals treated in local or adjacent herds, and the regularity of morning treatments. Observations made upon untreated animals in pastures near treated cattle revealed a significant reduction of flies on the untreated animals. For this reason, check animals used to establish a population index were at least 1 mile from the baited cattle.

*Toxicological Considerations.*—The amount of toxicant on each animal is so small as to be insignificant. Only 10 to 20 mg. dimethoate and 4 to 8 mg. of DDVP need be applied at 1- to 5-day intervals to give effective control. The mammalian acute oral LD50 of dimethoate is 245 mg./kg. and that of DDVP is 58 to 80 mg./kg. Each 500 kg. animal obtains a topical dosage of approximately 1/5,000 of the oral acute dosage. The amount of toxicant absorbed from the bait would be insignificant when compared with the quantity needed to produce toxic symptoms in skin absorption tests.

# Malathion Residues On and In the Leaves of *Phaseolus vulgaris*[1]

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ABSTRACT

By using P-32 labeled malathion, the speed of migration of malathion in the tissues of *P. vulgaris* L. was measured. Speed of migration was found to be very low in leaves. Malathion was taken up by the leaf tissues very rapidly, and the rate of entry of malathion into the tissue was found to satisfy Langmuir's adsorption equation. It was found also that the slow dissipation of malathion residues in the later stage (persistence stage) in this experiment was owing to the lower rate of evaporation effected by absorption of the insecticide by plant tissue.

The residual behavior of insecticides on and in the plant tissues is very important from an agricultural point of view, and has been studied by many researchers (Gunther & Blinn 1955, 1956). Residue degradation of malathion was studied by Smith *et al.* (1954) in lettuce and tomatoes, by Westlake & Butler (1953) in apple fruits, by Wetters (1959) in grain, and by Blinn *et al.* (1959) in peels of Valencia oranges. In these studies malathion was evaluated mainly by means of a colorimetric method (Norris *et al.* 1954), which does not detect the presence of malathion metabolites (Cook *et al.* 1958). In order to study the behavior of malathion as a physicochemical phenomenon, radioactive malathion was used in this experiment. By this method dissipation of malathion resulting from all phenomena other than chemical degradation can be observed. Gunther & Blinn (1955) have shown that certain insecticides show two steps of residual dissipation: initial rapid dissipation (degradation) and less rapid dissipation (persistence) in a later stage. These authors regarded the persistence phenomenon as caused by enzymic action or other metabolically induced alterations.

MATERIALS AND METHODS.—Radioactive malathion prepared (Casida 1958, O'Brien 1958) in acetone was deposited on the surface of a leaf of the bean by a microsyringe. Each drop was 0.04 mm.$^3$. The plant was allowed to stand under laboratory conditions for given periods. Then the portion of the leaf carrying malathion residue was cut off as a disc with a diameter of 1 cm. The disc was washed for 15 seconds with 10 ml. of acetone, to measure the surface remainder of malathion. The disc was then digested with 10 ml. of hot concentrated nitric acid in order to measure the amount of malathion adsorbed in the plant tissue. In all cases the amount of malathion was measured in terms of radioactivity as counts per minute (c.p.m.) by using a Geiger counter (Tracerlab SC-33A) and a liquid counting tube (M-6 20th Century Electronics Co.). In all cases counts were taken for 3 minutes.

To analyze the migration speed, radioactive malathion was injected into the stem at 3 cm. above the ground level. After 5 days the plant was cut into sections and each part of the plant was dissolved in 10 ml. of hot concentrated nitric acid to measure the migrated malathion in terms of its radioactivity.

RESULTS.—*Migration of Malathion in Whole Plants.*—The amount of the insecticidal residue relative to the plant tissue weight (p.p.m.) can be affected by evaporation, alteration of the material, plant growth, and migration in the plant tissues. If the migration speed is very high a large portion of the plant tissue must be taken to recover all the remaining insecticide. An experiment was conducted to measure the speed of translocation of malathion in plant tissues, and the result is shown in table 1.

**Table 1.—Migration of radioactive malathion in *P. vulgaris* tissues.**[a]

| PLANT TISSUES | RECOVERED MALATHION (COUNTS/MIN./GM. TISSUE) | NOTES |
|---|---|---|
| Root | 326.3 | |
| Stem (0–5 cm. above ground) | 2,791.7 | Injection point |
| Stem (10–15 cm. above ground) | 79.7 | |
| Stem (15–20 cm. above ground) | 101.0 | A branch connected to the growing point |
| Growing point | 167.0 | |
| Terminal leaf | 142.0 | One of the compound leaves |
| Simple leaf | 22.7 | |

[a] Migration for 5 days; temperature 23°–25° C.; under laboratory conditions

**Table 2.—Absorption of radioactive malathion by simple leaves of *P. vulgaris*.**[a]

| TIME AFTER APPLICATION (MINUTES) | C.P.M. MALATHION[b] | |
|---|---|---|
| | Remainder on the Surface | Absorbed by the Leaf |
| 0 | 88.3 | 18.7 |
| 10 | 69.3 | 26.0 |
| 40 | 77.0 | 29.0 |
| 100 | 79.7 | 30.0 |

[a] Temperature, 25.7° C.
[b] Averages of three replications.

It may be noted that migration of malathion in *P. vulgaris* was found to be very slow at the simple leaf. At the terminal leaves and the growing point, however, a high rate of migration was recorded, perhaps owing to the high rate of metabolism in the plant tissue. The speed of translocation then is not the same in different parts of the plant system, and the rate of migration is particularly low on leaves.

*Absorption of Malathion by Plant Tissues.*—Absorption in itself does not change the absolute amount of residue, but absorption affects the nature of the residue; and thereby may secondarily affect the total amount of residue. Results shown in table 2 indicate that malathion put on the surface of leaves cannot be thoroughly washed out by acetone even immediately after its application.

It seems that absorption reaches its initial saturation within 10 minutes after application. In order to characterize absorption of malathion by plant tissues, the rate of change of absorption was tested against concentration. The results shown in figure 1 are in accordance with Langmuir's adsorption equation (Langmuir 1916); in this

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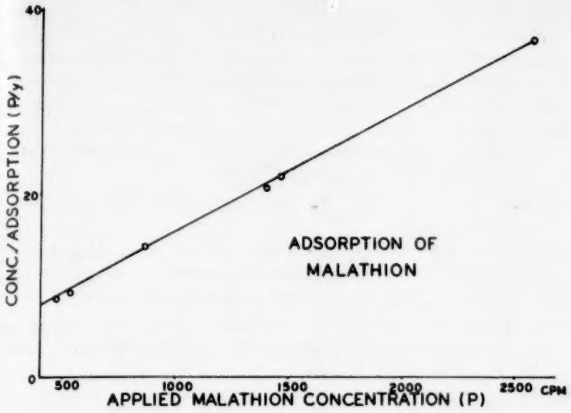


FIG. 1.—Absorption of malathion by *P. vulgaris* leaf tissues. (Application of Langmuir's equation to absorption of malathion for 2 hours at 23.7° C.; a plot of applied malathion concentration vs. the ratio of concentration to adsorption P/y should yield a straight line.)

type of adsorption the ratio of concentration to adsorption (conc./ads.) is assumed to be proportional to the concentration applied.

*Dissipation of Malathion.*—Types of dissipation of organic insecticides in plant tissues are illustrated by Gunther & Blinn (1955). Magnitudes of malathion residue on and in *P. vulgaris* leaves are shown in figure 2. First it can be seen that dissipation takes place very rapidly during the first 3 days (degradation) and then reaches a plateau (persistence). The characteristics of the curve are in accordance with the curve of Blinn *et al.* (1959). However the dissipation rate in the persistence stage in this experiment is found to be very low as compared with that found by Blinn *et al.* One of the most important reasons for lower rate of persistence is that the absolute amount of malathion was taken in this experiment instead of p.p.m., *i.e.*, this measurement changes as the plant grows. Clearly, then, dissipation of malathion in the persistence stage is not due to migration, metabolic alteration, or plant growth. The rapid degradation for the first 3 days is caused by evaporation of the initial deposit. In addition, it has already been shown that the slow dissipation (persistence) after 3 days is also caused only by evaporation of malathion and not by migration. In summary the amount of malathion left on the surface of the leaf, and therefore the amount available for evaporation, decreases rapidly at the first stage. The rate of evaporation after this first stage must decrease when most of the malathion is absorbed by leaves. The validity of this conclusion was demonstrated by comparing the data with those of pure evaporation. Drops of malathion with a volume of 0.04 mm.$^3$ were placed on a glass plate, and their evaporation was measured. The result is shown in figure 3. It can be that with time plant surface residue curves away from linearity, whereas with time the glass surface residue affords a straight line. The degradation slope is different in both cases owing partly to the different conditions, for example temperature, afforded by the glass and by the leaf. The main reason for the differences in the slopes is the gradual absorption of malathion by the leaf. That is,

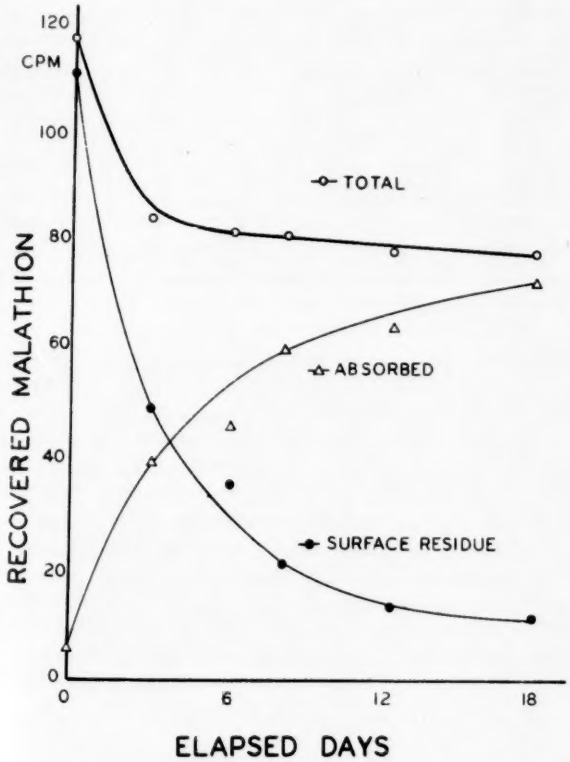


FIG. 2.—Residual behavior of malathion on and in *P. vulgaris* leaves in laboratory conditions.

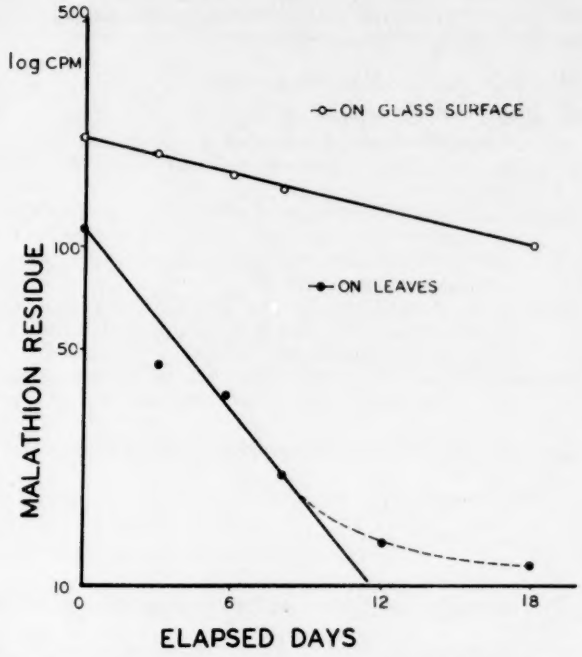


FIG. 3.—Evaporation of malathion from a glass surface and from *P. vulgaris* leaves. (Plotted in logarithmic coordinates: log. rate of evaporation against elapsed days should yield a straight line; temperature 24°–26° C., laboratory conditions.)

the steep degradation of the malathion residue on the leaf is attributable to absorption.

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# Evidence of the Oversummering of the Greenbug in the Texas Panhandle[1]

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### ABSTRACT

The greenbug, *Toxoptera graminum* (Rond.), was observed on five host plants not previously recorded. It was found to oversummer on western wheat grass, *Agropyron smithii* (Rydb.), during 1959. The aphid was observed in both the normal and the aestivating forms on this grass and migrated from it in the late summer to large volunteer wheat clumps and from those to wheat fields in the fall.


For many years it has been assumed that the summer survival of the greenbug, *Toxoptera graminum* (Rond.), is dependent upon hosts such as wild grasses and volunteer grains. Patch (1938) listed 62 grass plants on which the greenbug has been observed; however, she gave no information on the insect's reproduction ability and population maintenance on them. Dahms *et al.* (1954) subjected 23 grasses to greenbug infestation and found that only two failed to maintain a population. Eleven of these species were not recorded by Patch. Daniels (1956) mentioned the possibility that the egg was an oversummering stage in the Southern States when he observed greenbug eggs in the greenhouse. However, eggs have never been observed in Texas fields.

PRELIMINARY OBSERVATIONS.—Spot-check observations for greenbugs on various grasses have been made during the spring and summer of 1953–59. Efforts were not concentrated on this specific problem until the spring of 1959, when greenbugs were numerous in many areas of the State. Twenty species of grass on which greenbugs have been observed reproducing in the Panhandle area are listed in table 1. Five of these species were not mentioned by either Patch (1938) or Dahms (1954). Several of the 20 grasses are in the grass nursery at the Southwestern Great Plains Field Station. It was noted in 1957 that greenbugs maintained heavier populations on western wheat grass, *Agropyron smithii* (Rydb.), and remained later than on other grasses. Greenbugs had disappeared from most of the grasses by the last of May. Table 2 gives the dates when greenbugs disappeared from wheat in the spring and when they were found again in the summer or fall of 1953 to 1958 inclusive. Since 1953 greenbugs have been observed earlier each fall. This fact was probably due to gained observational experience of the author. During the late summers of 1956, 1957 and 1958 greenbugs were first observed on large volunteer wheat clumps growing around irrigation wells or ditches where moisture had been abundant during the summer. They later migrated from these clumps to the wheat fields. Greenbugs were seldom found on small wheat

**Table 1.—Grasses on which greenbugs were observed reproducing in the Panhandle of Texas, 1953 to 1959.**

| LATIN NAME | COMMON NAME |
|---|---|
| *Agropyron cristatum* (L.) Beauv.[a] | Crested wheatgrass |
| *Agropyron smithii* Rydb.[a] | Western Wheatgrass (Bluestem) |
| *Andropogon hallii* Hack.[a] | Sand Bluestem (Turkeyfoot) |
| *Andropogon ischaemum* L. | Yellow bluestem |
| *Bouteloua gracilis* (H.B.K.) Lag.[a] | Blue gramma |
| *Bromus catharticus* Vahl. | Rescue grass |
| *Bromus secalinus* L. | Cheat (Chess) |
| *Buchloe dactyloides* (Nutt.) Engelm. | Buffalo grass |
| *Cenchrus pauciflorus* Benth. | Field sandbur |
| *Cynodon dactylon* (L.) Pers. | Bermuda grass |
| *Dactylis glomerata* L. | Orchard grass |
| *Digitaria sanguinalis* (L.) Scop. | Crabgrass |
| *Elymus canadensis* L. | Canada wild-rye |
| *Elymus junceus* Fisch.[a] | Russian wild-rye |
| *Eragrostis curvula* (Schrad.) Nees. | Weeping lovegrass |
| *Eragrostis pilosa* (L.) Beauv. | India lovegrass |
| *Festuca elatior* L. | Meadow fescue |
| *Hordeum pusillum* Nutt. | Little barley |
| *Poa pratensis* L. | Kentucky blue grass |
| *Sorghastrum nutans* (L.) Nash. | Indian grass |

[a] Not listed by Patch (1938) or Dahms (1954).

[1] Technical Article No. 3393 of the Texas Agricultural Experiment Station. The author gratefully acknowledges the assistance of Mr. Joe C. Hill, Student Assistant, for his help in searching for aphids during 1959. Accepted for publication January 4, 1960.

Table 2.—Dates, latest in the spring and earliest in the summer-fall, when greenbugs were found on wheat, 1953 to 1958.

| YEAR | LATEST (SPRING) | EARLIEST (SUMMER-FALL) |
|---|---|---|
| 1953 | June 12 | October 23 |
| 1954 | June 15 | October 27 |
| 1955 | June 17 | October 11 |
| 1956 | June 11 | September 13 |
| 1957[a] | June 13 | August 26 |
| 1958 | May 19 | August 27 |

[a] Found on western wheat grass July 3.

during 1953–59 until after the first freeze or before the wheat stooled.

OBSERVATIONAL RESULTS.—During the spring of 1959, after greenbugs had reached damaging numbers in wheat, they were easily found on western wheat grass at the Bushland Station and in the Hereford-Dimmitt area. Greenbugs disappeared from the wheat by June 18 and were not found on wheat again until August 20, when they were found on large volunteer clumps near Hereford. However, greenbugs were observed periodically during the summer on western wheat grass in the aforementioned areas, table 3. They were not found at any location north of Amarillo during the summer. Greenbugs were more numerous during the summer on western wheat grass growing in locations such as bar ditches or around ponds. Such locations were better greenbug habitats when partially shaded. During July greenbugs became difficult to find in dry upland locations where the grass was extremely tough. August 28 was the latest date during the summer, that the aphids were found on western wheat grass. However, on that date they were also found on large wheat clumps. The greenbugs had apparently migrated from nearby western wheat grass to the volunteer wheat clumps.

During July small yellow aphids, which appeared to be the aestivating form of the greenbug, were observed on the western wheat grass. To determine whether or not these aphids were aestivating forms of *T. graminum* they were brought in from the field and reared on wheat in the insectary. The laboratory conditions were approximately 74° F., 40% to 50% relative humidity, with 16 hours of light. After these aphids were transferred to the insectary normal growth and reproduction resumed. Several normal greenbugs, found on the western wheat grass along with the aestivating forms, were also transferred to the insectary and reared through several generations. Both forms produced normal offspring, which were positively identified as *T. graminum*. Kring (1959) observed that the melon aphid, *Aphis gossypii* Glov., oversummered

Table 3.—Host plants and dates on which greenbugs were found during the summer of 1959 at Bushland, Texas.

| DATE | HOSTS | | |
|---|---|---|---|
| | Western Wheat Grass | India Lovegrass | Wheat |
| June 6 | X | | X[a] |
| June 18 | X | | |
| July 8 | X | | |
| July 21 | X | | |
| July 27 | X | X | |
| August 7 | X | | |
| August 18 | X | | |
| August 20 | X | | X[b] |
| August 28 | X | | X[b] |
| September 7 | | | X[b] |
| September 11 | | | X[b] |

[a] Drilled wheat, ripening.
[b] Large volunteer clumps.

in the yellow aestivating form on *Catalpa bignonioides* (Walt.).

During the summer of 1959 normal greenbugs taken from both the western wheat grass in the field and from wheat in the insectary were reared out of doors on wheat grown in pots. The greenbugs taken from the insectary were from insectary parental stock of many generations. The wheat plants were covered with plastic cages and the pots were kept in the shade on the north side of a building. Greenbugs from the two sources of infestation were reared in this manner from July 27 until the middle of October, during which time no aestivating forms appeared. This fact indicates that greenbugs may also pass the summer without going into aestivation.

Greenbugs have now been found every month of the year in the Texas Panhandle. In years of extreme drought they undoubtedly oversummer wholly in the aestivating form on western wheat grass. If during some years the aestivating forms completely disappear, thus breaking the life cycle, fall greenbug infestations will depend on wind migration from other areas.

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# The Effect of Grain Moisture Upon Development of the Rice Weevil in Green Corn[1]

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ABSTRACT

The rice weevil (*Sitophilus oryza* (L.)) is known to infest standing corn in the field in Louisiana. It was not known at what maximum grain-moisture level oviposition followed by complete development occurred. Studies conducted over a 2-year period showed oviposition may occur at a grain moisture of 65%. Eggs laid at this stage of corn maturity required 9 days for incubation. Complete development from egg to emergence of the adult required 42 days, and occurred between a grain moisture change from 65% to approximately 25%.

Considerable information relative to the effects of the moisture content in stored dry cearel grains on the development of the rice weevil (*Sitophilus oryza* (L.)) is available (Reddy 1950, Tsai & Chang 1935). The minimum moisture in grain at which this species can develop is considered to be approximately 9%. Reddy (1950) showed that the optimum moisture of stored dry grain for development of this species was 17.6%.[3]

In Louisiana, corn, stored in the shuck, is usually harvested when the grain moisture is between 20% and 15%. The moisture gradually decreases in storage until the following spring and summer, when it becomes stabilized at approximately 12% to 13%. Thus, the grain moisture of stored corn in Louisiana is adequate for development of the rice weevil throughout the storage period. Rapid increase of the insect is further augmented by favorable temperatures during part of the storage period.

Although much information concerning development of the rice weevil in dry corn at various moisture levels is available, no information has been published relative to the maximum grain moisture of the standing crop at which development can take place.

Corn grown in the lower South is nearly always infested in the field by the rice weevil (Floyd *et al.* 1959). It is commonly known that at least one generation is developed in the field before harvest. At what moisture level eggs for this generation were oviposited was not known. Adult rice weevils have been found in the field as early as April 30, feeding on strawberries, in the Baton Rouge area.

In order intelligently to plan control experiments designed to alleviate field infestation it was imperative to determine the maximum grain moisture of green corn at which the rice weevil may develop. In 1956 a study was begun by the Louisiana Agricultural Experiment Station at Baton Rouge to devise techniques to determine the maximum grain moisture at which it can develop. The greater rice weevil (*S. oryza*) was used in these studies. The lesser rice weevil (*S. sasakii* (Tak.)), although capable of breeding in corn, is not a corn pest in Louisiana.

1956 STUDY.—PROCEDURE. The technique that seemed most promising consisted of caging weevils on ears of corn at various moisture levels with 32-mesh Saran screen cages. Louisana 521, a white hybrid, was used in 1956. Immediately before caging an ear 10 previously mated adult female rice weevils were introduced inside the shucks surrounding the ear tip. The husks were separated at the apex and the adult weevils were placed on the tip of the ear. The husks were then folded back in position and tied loosely with a small cotton thread. After the cage was placed in position a band of cotton was placed between the bag and the ear shank and the bag tied tightly to the shank with a cord. This method prevented the escape of the introduced rice weevils and prevented the entrance of "foreign" rice weevils. The cage was removed after 7 days and the introduced weevils recovered and destroyed. Detection of all the introduced adults was usually not possible until some of the husks were separated. After removal of the adults the husks were pulled back into their normal positions and the cage replaced and securely closed. The caged ears were then left undisturbed until harvest. Introduction of adult rice weevils into test ears was made weekly. At each introduction 12 ears were infested and caged. The grain moisture was determined by direct distillation at each introduction period by using comparable ears from adjacent stalks. Adult weevil introductions were made at 78%, 65%, 55%, 43%, 37%, 33%, 28% and 26% grain-moisture levels. At the time of the first introduction (78% grain moisture) 25 additional ears were caged but were not infested with weevils. These ears were intended to serve as a check to determine the efficacy of the plastic screen bags in preventing weevil entry into the ears from the time of caging until harvest. It was assumed that at this stage of maturity (78% moisture) no eggs of the rice weevil occurred in these ears.

Several workers (Kyle 1918, Cartwright 1930, Eden 1952, Floyd *et al.* 1959) have cited the value of long, undamaged husks in preventing entry of the rice weevil into ears of corn. The ears used in the present study were selected on the basis of possessing long, tight and undamaged husks.

When the grain moisture of ears on adjacent plants reached 15%, ten of the caged ears from each date of weevil introduction were removed from the plants and examined for infestation signs and/or adult rice weevils. Larvae of the corn earworm emerging from ears to pupate cut through the Saran screen. Ears protected with damaged screens were not examined.

*Results.*—At harvest no signs of infestation or any adult rice weevils were found in the ears infested at the 78% grain-moisture level. If oviposition occurred the eggs failed to develop. Adult rice weevils and signs of larval infestation were found in the ears infested at the 65% grain-moisture level and in all subsequent levels. The degree of infestation increased for each date of weevil introduction through the 37% grain-moisture level. The degree of infestation gradually decreased at moisture levels lower than 37% (table 1).

[1] Accepted for publication December 14, 1959.

[2] Research Fellow in Entomology and Associate Professor of Entomology, respectively.

[3] It is not known with which species of rice weevil, *Sitophilus oryza* (L.) or *S. sasakii* (Tak.), these workers were concerned.

**Table 1.—Results of introducing 10 adult rice weevils to individual ears of corn at various grain-moisture levels, 1956.**

| Date Caged | No. Ears Caged | Per Cent Grain Moisture (when caged) | No. Days Before Examined[a] | Ears In-Infested (%) | No. Adults Found per Ear |
|---|---|---|---|---|---|
| June 29 | 25[b] | 78 | 92 | 0 | 0 |
| 29 | 10 | 78 | 92 | 0 | 0 |
| July 6 | 10 | 65 | 85 | 20 | 0.4 |
| 13 | 10 | 55 | 78 | 20 | 0.6 |
| 20 | 10 | 43 | 71 | 50 | 2.4 |
| 27 | 10 | 37 | 64 | 100 | 9.9 |
| Aug. 3 | 10 | 33 | 57 | 80 | 7.6 |
| 10 | 10 | 28 | 50 | 50 | 3.2 |
| 17 | 10 | 26 | 43 | 50 | 2.3 |

[a] Ears were pulled and examined when the grain moisture reached 15%.
[b] Uninfested check.

*Discussion.*—Since the adult rice weevils were allowed to remain inside the cages for 7 days following introduction, the eggs could have been oviposited at any time between introduction and removal of the adults. The grain moisture of the ears infested at the 65% moisture level decreased to 55% seven days later. Therefore oviposition may have occurred sometime within the 7-day period at a grain-moisture range between 65% and 55%.

Since the degree of infestation increased as the grain moisture decreased to the 37% level and decreased thereafter as the grain moisture decreased, it is indicated that the optimum grain moisture of the corn for development of the rice weevil is approximately 35%.

1958 STUDY.—Field studies were continued in 1958 employing similar but modified techniques to determine more accurately the grain-moisture level at which oviposition, followed by complete development of the rice weevil, may occur. Changes in techniques used in the 1958 study were designed to eliminate the possibility of any natural infestation in the ears and to allow for more frequent introductions of adult weevils.

*Procedure.*—A uniform planting of Funks G-134, an early maturing yellow corn hybrid, was made in 1958 on 42-inch rows with single plants spaced 24 inches apart. Immediately after pollination was apparently completed (June 11) randomly selected top ears were placed in cages in a manner similar to those described for the 1956 study. The ears were caged at this early stage of development, when the kernels were immature, to lessen the possibility of any natural infestation. These caged ears were to supply adequate samples for weevil introduction, grain-moisture determinations, and examination at periodic intervals to determine the developmental stages of the rice weevils. Plants bearing the caged ears were selected on the basis of uniformity in size, maturity, and spacing in the row.

On June 19, when the grain moisture was 75%, the screen cages were removed and each ear infested with 25 female and 10 male adult rice weevils. The husk tips, as

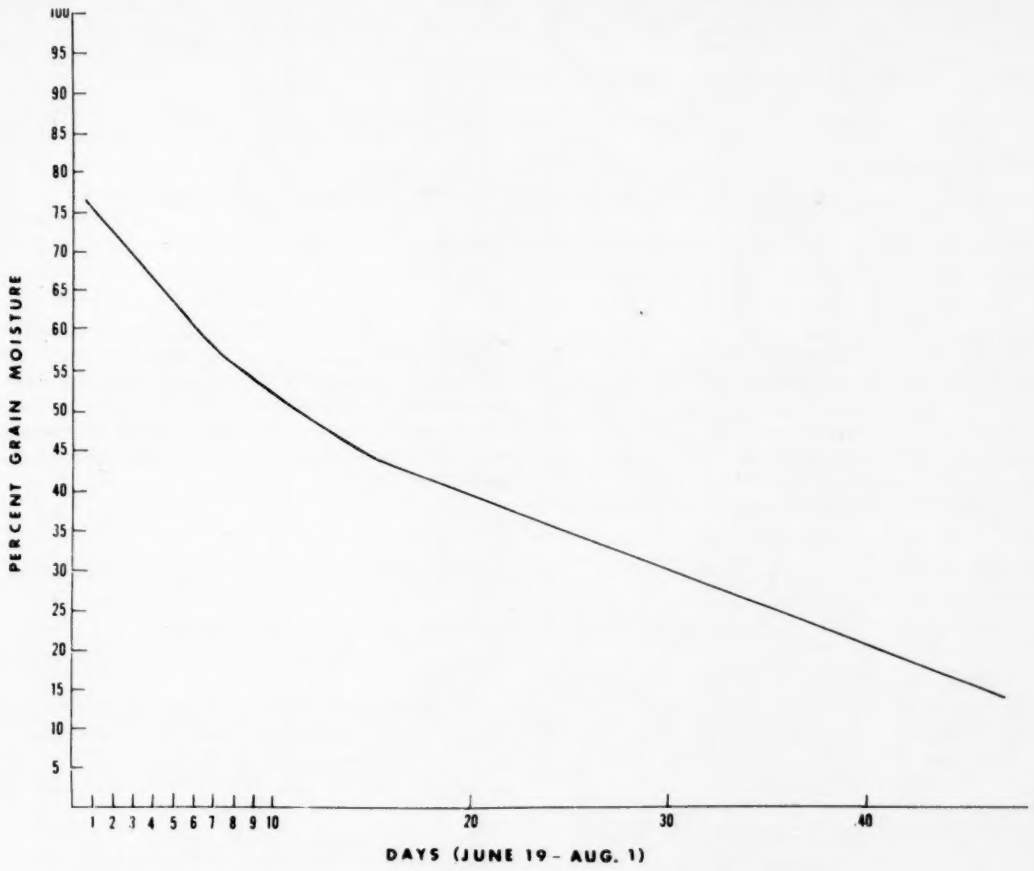


FIG. 1—Loss of grain moisture from green corn, Baton Rouge, 1958.

previously described, were separated sufficiently to permit entrance of the insects inside the ear tip, then closed and the plastic screen cages replaced.

In addition to the ears infested with adults at the 75% moisture level, similar introductions were made in previously caged ears at 65%, 58%, 51%, 42%, 41% and 35% grain-moisture levels. Ten ears were used for each of the seven introductions. The Saran screen cages in all introductions after the first were replaced with cones made of ¼-mesh hardware cloth screen, which fit snugly over the ears. These wire cones protected the ears from birds and rodents.

The grain moisture was determined at the time the first adult rice weevils were introduced and every 3 or 4 days thereafter for a period of approximately 40 days (fig. 1).

Daily, beginning the second day following the introduction of the adults into ears with grain moisture of 75%, one ear was removed and taken to the laboratory, where it was shucked and the kernels examined under a magnifying lens for rice weevil eggs and subsequent stages. On the date the first eggs were found in ears exposed to infestation at the 75% grain-moisture level, examination of these ears for eggs was made. Remaining ears were periodically examined to ascertain the stages of the developing weevils present.

*Results.*—Examination of the ears infested at the 75% grain-moisture level showed that oviposition occurred the third day after introduction of the adult weevils. The grain moisture at this time was 65%. The first newly hatched larvae were found on the ninth day after the first eggs were discovered. At this time the grain moisture was 48%. The first pupa was found on the 37th day following oviposition, and the first adult emergence occurred on the 42nd day following oviposition. Complete development from date of oviposition to emergence of the adult required 42 days, and occurred between a grain-moisture change from 65% to approximately 25%.

Grain-moisture determinations made on comparable uncaged ears during the course of the study showed that the presence of this type of screen cage over the ear did not influence the grain moisture at any time during the development of the ear from the premilk stage to the early harvest stage. Daytime temperatures taken inside the ear surface of caged ears and of comparable uncaged ears were identical.

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# Forest-Insect Surveys in the Central Rocky Mountains[1]

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ABSTRACT

With the growth of forestry, survey methods have been developed to detect and appraise forest-insect infestations. Detection is made by the discovery of an outbreak through field surveillance or by means of systematic aerial and ground surveys. Following the discovery of an outbreak, an inspection of the infestation is sometimes made by an entomologist to determine the need for an appraisal survey. The appraisal survey is divided into two phases: (1) an entomological measurement of the potential threat of the infestation, referred to as a biological evaluation; and (2) a systematic cruise of the area to obtain information for control planning. The objectives of the program are to allow no outbreaks to escape early discovery, and upon discovery, to obtain information about the insect concerned—where it is, what it is doing, its potentialities, methods for control, and data directly concerned with control.

Forest insect surveys in the central Rocky Mountains are important in forest protection, yet not all foresters and entomologists fully understand the meaning of the term.

WHAT ARE FOREST-INSECT SURVEYS?—Forest-insect surveys are procedures for detecting and measuring insect infestations of known or of potential importance in forested areas. These forests may be aesthetic settings for picnic areas, habitats for game animals, or sources of timber for farmers' barns or for city housing developments. Although many insect species are considered in surveys, those that injure and kill forest trees are generally most intensively considered. The seed eaters, root feeders, stem girdlers, bud infesters, leaf chewers, and wood borers are the insects that destroy trees, whether the trees are in a flourishing nursery, in a thirfty stand of poles, or in a scenic wilderness area, and regardless of ownership.

Forest damage, however, is caused by a relatively few native insect species and fewer introduced ones. Native species have been with us a long time, but introduced insects sometimes become as well established and even

[1] Accepted for publication December 14, 1959.

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more destructive. They are all part of the forest environment. Occasionally, one of these insects tends toward self eradication by destroying its food supply. This self eradication almost happened a few years ago when one bark beetle, the Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk. killed much of the Engelmann spruce stand of Colorado and threatened the remainder. However, control by parasites, predators, weather, and direct attack by man terminated the epidemic.

Surveys were part of that control. Forest-insect surveys determine the presence of insects and their effects on host plants, measure their abundance, delineate their boundaries of destruction, measure the environmental effect, and predict their trends. Surveys provide the basis for combating insect pests.

The purpose and the organization of forest insect surveys in the United States were spelled out in June 1947, when Congress legislated that, " . . . it shall be the policy of the Government of the United States independently and through cooperation with the Governments of States, Territories, and possessions, and private timber owners to prevent, retard, control, suppress, or eradicate incipient, potential, or emergency outbreaks of destructive insects . . . on, or threatening, all forest lands irrespective of ownership." Congress authorized the Secretary of Agriculture " . . . to conduct surveys on any forest lands to detect and appraise infestations of forest insect pests . . . , to determine the measures which should be applied on such lands in order to prevent, retard, control, suppress or eradicate incipient, threatening, potential, or emergency outbreaks of such insect . . . pests. . . . "

Thus, the Pest Act of 1947 clearly defined for the first time the responsibility of the U. S. Department of Agriculture for protecting the Nation's forest resources from insect pests. As a consequence, it has enabled the Department's forest entomologists to recognize and direct their survey activities much more productively than would otherwise have been possible. Although the federal forest entomologists were now responsible for detecting and measuring infestations, valuable contributions, especially in discovering incipient outbreaks, still would be made by the many cooperating persons. After all, the forested areas in the central Rocky Mountains of Colorado, Wyoming, and South Dakota gross more than 27 million acres and involve many ownerships.

Specifically, the objective of detection is to discover outbreaks and potential outbreaks of destructive insects. Detection can be either by casual observation or by a planned survey.

FIELD SURVEILLANCE.—Field surveillance, the incidental observation of insect activity or damage, may be done by anyone. Such observations are made both by persons trained in the technique of detection and by persons as inexperienced as one who spots a forest fire and reports it to the nearest forest ranger or to the county sheriff. Many observations of insect outbreaks are reported by members of the Forest Service, Park Service, and Bureau of Land Management. State foresters, timber operators, ranchers, sportsmen, and picnickers also report their observations.

DETECTION SURVEYS.—Detection surveys, on the other hand, are planned or are systematic inspections of forest areas, such as those carried out by the Forest Service during its annual aerial insect surveys (Fig. 1). Detection surveys by air observers or by ground crews are the designated responsibility of the Forest Service on lands under its own administration. On other lands, discovery of infestations is the responsibility of the respective owners or managers, in cooperation with the Forest Service.

FIG. 1.—Aerial view of a quarter-section of blowdown in spruce type, San Juan Mountains, Colorado.

Detection also requires the preparation of a report for an official qualified to judge its significance. Then a forester or entomologist visits the area (Fig. 2) to determine whether control or further evaluation is necessary.

Evaluations are usually restricted to infestations of important injurious insects that cause economic damage. When a market develops for a tree species that currently is of little or no commercial value, such as subalpine fir, the insect pests of that tree will be dealt with. Likewise, when the public demands, the insects that threaten trees of aesthetic value will be evaluated.

After detection, an evaluation of an infestation includes: (1) A biological evaluation of the insect population and its trend; (2) a systematic cruise to delineate and determine the magnitude of the infestation before applying direct control; (3) and a post-control survey to measure the effectiveness of treatment.

If the biology of the insect is known, if procedures for determining its population trend are available, and if field experience is sufficient, an evaluation can readily be made. Those three catalogued requirements, however, have been developed for only a few insects. No two insects

FIG. 2.—For spring inspections a sno-cat is occasionally needed to reach bark beetle infestations, as here in a lodgepole pine-spruce stand on the Continental Divide in Colorado.

are alike. Fortunately, though, similarities among kinds of insects (*e.g.*, among bark beetles, among sawflies, or among tent caterpillars) do occur. Hence, evaluations of the infestations of lesser-known insect species can be made by applying reason and judgment based on an understanding of the better-known related ones.

BIOLOGICAL EVALUATION.—Biological evaluation of an infestation is based on many factors, both independent and interrelated. Obviously, the destructive insect and its host must be known. The damage, by kind and degree, or threat of damage must be known. Relationships between the pest insect and its associates must be considered. Kinds and abundance of insectivorous parasites and predators, other predaceous animals, and insect diseases are also biological factors. Correct timing of the evaluations is essential if natural control factors are to be measured when they are most critical (Fig. 3). The effect of other factors, such as weather and climate, site, and stand composition must be considered. In fact, all information needed for a good description of the infestation, its course, and the expected results of control must be gathered.

The responsibility for making biological evaluations rests directly with the Forest Service if the infestation is on Forest Service or other Federal lands; otherwise the Forest Service is responsible only for aid with the evaluations.

PRESUPPRESSION SURVEY.—The report of each biological evaluation is basic to the forest manager's planning for control. This report provides management with information on the insect involved, the timber type and particular host plants under attack, as well as the location, topography, accessibility, and the recommended control. Should the forest manager decide that control will be carried out, he makes a systematic cruise of the area of infestation to determine the size of the job ahead. This cruise invariably involves a preliminary step called scouting, a cursory, overall examination to delineate the area of damage and to relate it to the endemic areas. The variables and combinations of variables presented by insects and insect damage preclude a cut-and-dried cruise technique. The habits of each insect species, host tree, and environment determine the cruise procedure (Fig. 4). The systematic cruise determines acreage of infested host type, its distribution, and its relationship to other forest types and to topography. The magnitude of the outbreak prescribes the job of (1) recruiting, training, housing, feeding, and transporting project manpower; (2) acquiring project material; (3) locating and establishing bases of operation; (4) timing; and (5) applying control.

A post-control survey determines the effectiveness of the control job and collects information for a report on accomplishment. This systematic cruise is much like that made for planning control. If needed, it can also be used for planning follow-up control.

AN INCIDENT FOR EXAMPLE.—One forest-insect survey was recently begun as the result of a report from a trout

FIG. 3.—Examining insects in bark of beetle-infested spruce, Routt National Forest, Colorado.

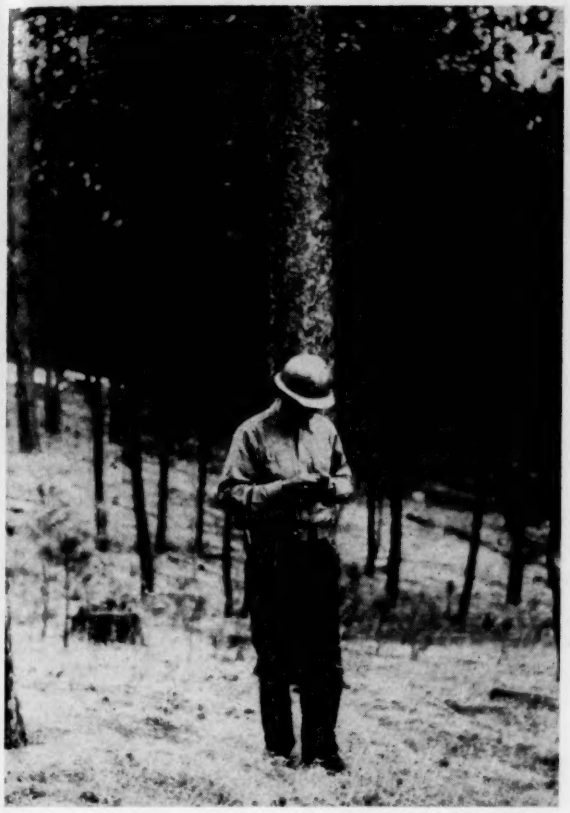

FIG. 4.—By hand-compass and pacing, a bark beetle-infested area of ponderosa pine is surveyed in the Black Hills of South Dakota.

fisherman who packed into a remote wilderness area. The fisherman had returned to an old haunt after an absence of 3 years and found his familiar camping spot changed. All the lodgepole pines were dead, a few nearby had off-color foliage. He also found "pitch-tubes" on the bark of the faded trees and many bark beetle larvae under the bark.

On his return to civilization, he reported his observations to the U. S. Forest Service district ranger, who in turn examined the infestation and reported to his regional pest-control office. The control office requested the entomologists at the Forest and Range Experiment Station to visit the area and evaluate the biological significance of the outbreak.

The survey revealed that the mountain pine beetle, *Dendroctonus monticolae* Hopk., had built up to an epidemic level. Infested trees selected at random within the areas were systematically examined. Two bark samples, each 6 inches square, were cut from the boles of each of 25 trees at a convenient height of 4 to 6 feet above the ground. The living bark beetles and associated insects were counted. In this instance, the bark beetles averaged 47 per square foot, but almost no parasites or predators were present. The trend of the infestation was upward; the outbreak was getting worse. If direct control could be started by the following spring, a valuable stand of lodgepole would be saved. Without control, the rest of the type contiguous to the infested block might be lost. Hence, methods and techniques for control were advised in a report, along with procedures for mixing and applying an insecticide.

Officials in charge studied the report and decided to control the outbreak. First, they made a systematic survey, the prerequisite to control planning. The infested wilderness area was accessible only by foot or by horseback. Packing in and out required two saddle horses and one pack horse, and took 2 days. After the two-man crew reached the outbreak, they completed the systematic survey in 3 days. Tools for the job included a map and aerial photographs, hand box-compasses, hand axes, and tally sheets. The men also had to pack camping equipment and food.

Extent of infestation and intensity of infestation warranted a $2\frac{1}{2}\%$ survey. This particular survey is a cruise to assure a satisfactory estimate of the infestation for control planning. Experience and requirements dictated that the survey be made by tallying infested trees on strips one-half chain wide spaced 20 chains apart.

The infestation was confined to one drainage in rough topography. It was a long, narrow, 6 mile strip along a south-flowing stream. Aerial photographs showed that the lodgepole type extended no more than one-half mile on either side of the stream. Cruise lines were plotted on the aerial photographs. Starting at the streamside trail as a base line, one member of the crew surveyed east of the trail; the other cruised the west side. Each man, having selected his first line at random within the first 10 chains, cruised on a compass bearing at right angles to the base line, to the edge of type, then offset upstream 20 chains, cruised on the line parallel to his first line, to the trail, offset up the stream and so on until the cruise was finished about the middle of the second day.

The tally sheets were set up to record both last year's and the current year's beetle-killed trees and the surviving uninfested lodgepoles. Tallies, of course, were made along the cruise lines, one-quarter chain on each side; no tally was kept along the offset lines. Either at camp or after returning to headquarters, the data were tabulated. The resulting estimate, $6{,}722 \pm 972$ currently infested trees, statistically accurate within an acceptable percentage of error, was incorporated in a formal report. The report further revealed that the infestation had jumped to the current number of infested trees from $4{,}225 \pm 881$ the year before. Thus a series of surveys was completed.

ANOTHER INCIDENT.—On a routine aerial detection survey the observer found a block of defoliated quaking aspen on Forest Service land that had escaped earlier surveillance. The 800-acre block of second-growth aspen on a south-facing slope was only 2 miles south of a main public highway but hidden by a ridge.

The observer landed 30 miles from the infested area, drove to within a mile of the area and walked in. He confirmed his recording of heavy (complete) defoliation by the Great Basin tent caterpillar, *Malacosoma fragile* (Stretch). The insects had already pupated; there had been no excessive caterpillar mortality. The infestation was reported and the regional pest control officer requested a biological evaluation. An entomologist completed the evaluation in 1 day, assessing both the forest stand and the insect.

The trees were well formed, 6 to 8 inches in diameter,

without limbs to 20 feet, and about 40 years old, a good aspen site. The area had supported spruce before it was burned years ago.

The egg masses on the branches of five randomly selected trees were counted. They averaged more than four current and less than two old egg masses per branch. The infestation was not new; it had simply escaped earlier detection. The narrow annual rings for the past several years was further evidence that the infestation was not new. Although no tree mortality had resulted, a prolongation of the outbreak might severely cripple the stand. Some egg masses were taken to the laboratory for observation. Many caterpillars but few parasites emerged. The large number of egg masses and viable eggs forecast severe defoliation. Direct control by air application of insecticide would terminate the outbreak.

The decision by officials to control this infestation required only the information thus made known by the detection report and the report of the biological evaluation, plus available maps and photographs. Another survey was completed.

These two cases exemplify different procedures for surveying forest-insect infestations. Whether it be bark beetles, sawflies, or caterpillars, the procedure is determined by the incident at hand. There are similarities but also variabilities in field applications. Forest-insect surveys have their ramifications; but they form a most interesting occupation.

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# Measuring the Surface Area of Apples for Expression of Pesticide Residues[1]

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ABSTRACT

Several methods of measuring the surface area of apples have been reported in the literature, but with little supporting data for their selection or by which to judge the magnitude of the errors associated with these methods. The present study was undertaken to evaluate the accuracy and precision of the use of weight, volume displacement, and diameter using planimeter measurement as a standard for determining the surface area of apples.

Because methods of calculating surface area assume apples to be perfect spheres, ping-pong balls were used as working standards. McIntosh and Delicious apples were sampled from two areas in each tree through the season for measurement and supplemented by weight and volume displacement data collected routinely from apple residue studies in previous years.

All methods used were found comparable on ping-pong balls. Surface areas of apples computed from volume displacement and the planimeter were not significantly different. Location of the fruit in a tree, development of the fruit during the season, and the variation from year to year were found to affect specific gravity significantly. Surface areas using weight and a constant specific gravity were significantly different from planimeter values on Golden Delicious but not on McIntosh. Both volume displacement and the weight method were superior to the others studied. Sample size, sources of variation, and bias were evaluated and are discussed.

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Although pesticide residues of apples are usually expressed on a p.p.m. basis, it is sometimes desirable to express the results on a surface-area basis. In studying insecticide deposition and weathering of residues of different formulations, small differences may require the use of a precise method of determining the surface area of a sample of apples. Also it is the only common basis for comparing foliage and fruit deposits with each other, and with other crops or treated surfaces in toxicity and residue studies.

It is common practice to calculate the surface area of an apple on the assumption it is a sphere. Magness *et al.* (1926) used measurements of the circumference, while Hamilton (1929) used caliper measurements of the diameter of tagged apples. Others measured the transverse and vertical axes and used the average of these diameters to calculate the surface area (Smith 1926, Baten & Marshall 1943, Gunther 1948). Chapman *et al.* (1934) measured the volume displaced by an apple to calculate the diameter of a sphere of that volume and then used the diameter to compute the surface area. Norton & Billings (1941) used measurements of the volume displaced in the formula $4.84\sqrt[3]{(v/n)^2}=\text{cm.}^2$ to calculate the surface area of a sample of apples, where $v$ is the volume displaced and $n$ the number of apples in the sample.

The assumption of a constant volume displaced (1.2 cc.) per gram weight was used by Carter & Newcomer (1933) to determine the surface area with the formula $4.84\sqrt[3]{(\text{wt.}\times 1.2)^2}$. This use permitted the expression of residues as p.p.m. or as micrograms/cm.$^2$ with a single measurement of weight. Fahey & Rusk (1937) in their earlier work obtained the average weight and specific gravity of a representative sample, but later (1940) used the formula of Carter & Newcomer (1933). Huelin & Gallop (1951) used a factor of 1.028 to correct the surface area of mature Granny Smith apples computed from the volume displaced.

Barnes (1929) considered an apple as a cardioid and used the formula $0.755\sqrt[3]{(\text{wt.}\times d)^2}$ where wt. is the weight of the apple in grams and $d$ the specific gravity to estimate the surface area in square inches. He reported that the surface areas computed by this formula did not differ

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over 5% from his best "unstated" mechanical measurements. Groves & Marshall (1935) suggested what they believed to be an improvement on Barnes' method in that the area of the surface of the revolution of a cardioid is equal to ($p=a(1-\cos\theta)$ which is approximately $20.1\times a^2$, where $a$ is determined by multiplying the diameter perpendicular to the core by 0.385 or the diameter parallel to the core by 0.444.

Baten & Marshall (1943) determined the surface area of several varieties of mature fruit by tracing outlines of thin slices of narrow longitudinal sections of the apple on paper and then measuring the area of each section with a planimeter. They also measured various diameters, circumferences, and the weight of each apple and then regressed these values on the surface areas measured with the planimeter to determine the linear function, $a+bx=y$, or $bx=y$, where $x$ is the measurement, $a$ the intercept of the $y$ axis and $y$ the surface area. They concluded their regression formulas (one for each variety) for weight to be most satisfactory.

Others have determined the surface area of a portion of the apple. Markely & Sando (1931) used disks cut from the fruit surface with a cork borer and calculated the area of the disk by use of the formula for determining the area of the curved surface of a zone of one base. Brown (1951) cut cross-sectional slices from apples and computed the surface area of each sectional slice. These methods can be used to measure limited areas accurately, but they are not generally satisfactory where total surface area, or where growth of the surface area, is of interest as in residue studies.

Although much work appears to have been done on this subject, little data or documentation has been presented in support of the conclusions reached, or by which to judge the errors associated with the use of these methods. The purpose of this study was to determine the accuracy and precision of surface areas computed from the volume displaced and from the weight assuming the apples to be spheres, and to compare these methods with others reported in the literature.

MATERIALS AND METHODS.—In 1958, four McIntosh and four Golden Delicious apple trees were selected from a row of approximately 16-year-old trees in the Cornell Orchard at Ithaca, New York. Both varieties carried a larger than average crop. Apples were sampled from these trees periodically between June 30 and September 2, 1958. One apple was selected from the outside and one from the center of each tree and labeled. Measurements of weight, volume of water displaced, longitudinal and transverse diameters, and surface area with a planimeter were made.

After removal of stems, weights were measured to the nearest 0.1 gram. The volume of water displaced was determined by inserting a probing needle in the stem end of each fruit, submerging the apple in a beaker of water containing 2 drops of Triton X-100[3]/liter, and catching the overflow in a graduated cylinder. Repeated determinations of the volume displaced gave values within 1 ml. To calculate surface areas, Norton & Billings' (1941) formula using volume displacement and Carter & Newcomer's (1933) formula using weight were used.

The apples were then cut in half and the longitudinal and transverse diameters measured to the nearest 0.5 mm. The stem-calyx axis was measured from the point of stem attachment to the base of the calyx lobes. The hollow area enclosed at the calyx end of Golden Delicious was not considered as part of the surface area. The transverse axis was the greatest diameter across the cheeks of the apple and usually at right angles to the longitudinal axis. The average of the two diameters was used to compute surface area.

The skin was then cut radially from stem to calyx into 11 to 14 sections, and placed in a distilled-water solution of 1.6% ammonium oxalate and 0.4% oxalic acid for 24 to 48 hours (Huelin & Gallop 1951). The skin from each section was then removed and flattened on an index card while the card was held taut over the curved surface of a vial or jar of similar size to the fruit, with as little stretching, breaking, or wrinkling of the skin as possible. The outline of each section was traced immediately while held in this curved position. The cards were labeled and the surface area of each section determined using a compensating polar planimeter with an adjustable tracer arm.[4]

Maximum movement of the planimeter carriage was obtained by moving the carriage near the tracing point, thus maximizing any error in tracing an outline. Each outline was circumscribed once and the value noted. The outline was then circumscribed three more times and if the first value, when multiplied by four, did not agree with the final value within 0.1 cm.[2] the entire process was repeated until the values did not differ by more than this amount.

To estimate the precision (the ability to reproduce results) and accuracy (to measure the true value without bias) of the techniques employed, the surface area of each of four new ping-pong balls was computed using the diameter, the volume displaced, and planimeter measurements.

In addition, use was made of volume displacement and weight records of samples taken from orchard-insect experiments for insecticide residue studies during 1956 and 1957. One-fourth of each of these samples was taken from the center and three-fourths from the outside periphery at a 4- to 6-foot height, by clipping the apples directly into a tared gallon jar. The number of fruit per sample varied from 50 to as few as 10, depending on the stage of development and the number required to fill a wide-mouth 1-gallon jar. The weight of each sample was recorded to the nearest gram. After extraction of the insecticide, the volume displaced by the entire sample of fruit was measured and the surface area computed using the formula of Norton & Billings (1941).

RESULTS.—The surface areas of each sample of apples computed from the average planimeter values, average volume displaced, average weight using a specific gravity of 0.833, average of the mean of the horizontal and transverse axis, and the average weight using Baten and Marshall's regression formulae, are presented in table 1.

The specific gravities of these same apples along with a statistical analysis are presented in table 2. A detailed study of specific gravities of McIntosh apples calculated

[3] Rohm & Haas Company, Philadelphia, Pa.
[4] Model No. 4240, Keuffel and Esser Co., New York, New York.

**Table 1.—A comparison of average surface areas (cm.²) of McIntosh and Golden Delicious apples on four sampling dates by five methods of determination. Cornell orchard, 1958.**

| Variety & Date | | Planimeter | Volume Displacement as Individual[a] Apples | Volume Displacement as Total[b] Samples | Weight[c] | Average Diameter | B&M's Weight[d] |
|---|---|---|---|---|---|---|---|
| *McIntosh* | | | | | | | |
| 1958 | 6-30 | 29.81 | 27.81 | 27.96 | 29.47 | 23.64 | 56.47 |
| | 7-15 | 47.80 | 48.40 | 48.62 | 48.79 | 38.39 | 68.94 |
| | 7-30 | 68.89 | 69.01 | 69.57 | 69.62 | 54.59 | 84.59 |
| | 8-15 | 73.89 | 79.88 | 80.21 | 80.18 | 61.52 | 93.61 |
| | 9- 2 | 104.02 | 104.66 | 105.85 | 102.29 | 72.03 | 114.87 |
| 1959 | 8-11 | 111.43 | 111.87 | 112.17 | 107.25 | | |
| | 9-14 | 137.59 | 140.57 | 141.36 | 133.86 | | |
| *Golden Delicious* | | | | | | | |
| 1958 | 6-30 | 25.87 | 24.47 | 24.67 | 25.45 | 22.01 | 51.86 |
| | 7-15 | 46.43 | 46.70 | 47.09 | 46.39 | 38.30 | 64.21 |
| | 7-30 | 61.94 | 60.43 | 61.01 | 60.67 | 48.82 | 74.69 |
| | 8-15 | 80.81 | 79.90 | 80.54 | 80.19 | 62.91 | 90.81 |
| | 9- 2 | 110.72 | 108.70 | 109.68 | 106.32 | 81.32 | 115.60 |

[a] Computed as $\frac{\Sigma(4.836\sqrt{V^2})}{N}$, where $N=8$, or the number of McIntosh or Golden Delicious apples for each sampling date, and $V$ the volume displaced by each apple.

[b] Computed as $4.836\left(\sqrt[3]{\frac{\Sigma V}{N}}\right)^2$

[c] Carter and Newcomer's formula $4.84\sqrt[3]{1.2\times wt.^2}$.

[d] Baten and Marshall's formula for

McIntosh $(0.129\ wt.+7.122)\ 6.452$
Delicious $(0.129\ wt.+6.623)\ 6.452$

where wt. is the average value for the sample in grams, and 6.452 the conversion factor for square inches to square centimeters.

**Table 2.—Average specific gravities of McIntosh and Golden Delicious apples.[a]**

| Location | Sampling Dates: June 30 | July 15 | July 30 | Aug. 15 | Sept. 2 | Average |
|---|---|---|---|---|---|---|
| *McIntosh* | | | | | | |
| Sun | 0.876 | 0.823 | 0.843 | 0.831 | 0.795 | 0.834 |
| Shade | .959 | .872 | .831 | .843 | .813 | .864 |
| Average | .918 | .848 | .837 | .837 | .804 | .849 |
| *Golden Delicious* | | | | | | |
| Sun | 0.895 | 0.822 | 0.834 | 0.842 | 0.797 | 0.838 |
| Shade | .883 | .823 | .856 | .833 | .815 | .842 |
| Average | .889 | .822 | .845 | .837 | .806 | .840 |

Analysis of Variance

| Source of Variation | d.f. | Mean Square[b] | F |
|---|---|---|---|
| Total | 79 | | |
| Dates | 4 | 20.675 | 14.56** |
| Varieties | 1 | 1.593 | 1.12 |
| Positions | 1 | 5.729 | 4.03* |
| Varieties×positions | 1 | 3.445 | 2.43 |
| Varieties×dates | 4 | 1.160 | 0.83 |
| Positions×dates | 4 | 0.799 | 0.56 |
| Positions×dates×varieties | 4 | 2.442 | 1.72 |
| Residual (pooled interactions) | 60 | 1.420 | |

[a] Single fruit sample from two portions of each tree, four trees on five sampling dates.
[b] All values multiplied by 1,000 to eliminate zeros.
* Significant at 5% level.
** Significant at 1% level.

from measurements taken during 1957 are presented in table 3 with statistical analysis. Additional specific gravity values found with McIntosh apples during 1956 and 1957 are given in table 4. A statistical summary of the surface area measurements computed by planimeter, volume displaced, and weight methods are presented in table 5.

DISCUSSION AND CONCLUSIONS.—The only method of actually measuring the surface area of an apple is to project the surface of the apple upon a plane surface and to measure the projection with an instrument, such as a planimeter, or if the projection of the apple is on a surface of uniform thickness the projected area can be cut out and weighed. With the methods used in this study, it was estimated that the error in removing the skins, projecting the surface of the apple onto a flat surface and in tracing and measurement was less than 0.5% of the total surface area.

*Precision of Methods.*—The determination of the surface area of four pig-pong balls provides some estimate of the precision of the methods and techniques employed. The diameter of the pig-pong balls was 3.80 cm. A sphere of this diameter has a surface area of 45.37 cm.². The average of the planimeter values for these same balls was $45.35\pm.84$ cm². The average volume displaced by these same balls was 27.49 ml. giving a surface area of 44.42 cm². Thus, these methods are capable of obtaining comparable results, though the degree of precision suggested may be greater than can be obtained in measuring apples.

*Volume Displacement Method.*—A sphere has the smallest surface area for a given volume; therefore, the greater the deviation from a sphere, the greater the surface area will be for that volume. With apples, it was postulated that the volume displacement method would give smaller values than the planimeter method and that the difference between the values recorded from these two methods would increase as the deviations from a sphere increased. In checking this postulate, it was found that the surface areas of McIntosh computed by the volume displacement method were larger than the planimeter values on four out of five of the sampling dates. With Golden Delicious the converse occurred. However, these differences were generally small and variable with the exception of the August 15 sample of McIntosh.

The surface areas by all methods were recomputed and agreed within rounding errors. An examination of the surface areas computed by the weight method showed general agreement with the volume-displacement surface areas, suggesting that the discrepancy may be associated with the planimeter measurement. To further check the August 15 discrepancy and to check the McIntosh values, since they did not conform to theory, additional samples were selected from the same trees on August 11 and September 14 of the following year. The surface areas were computed[5] for each of these apples as in 1958. These results are presented in table 1 along with the 1958 data. In both of the 1959 samples, the volume-displacement method gave larger surface areas than the planimeter method, indicating a possible varietal characteristic, but the differences are smaller than the August 15, 1958, sample.

Statistical analysis of the 1958 and 1959 data (table 5) showed that the volume-displacement method signif-

[5] The authors acknowledge the assistance of Bruce L. Parker and D. N. MacDougal in making these measurements and calculations.

**Table 3.—Specific gravities of McIntosh apples showing the variation within and between trees with sources of variation.[a]**

| Tree Number | 8-6 | 8-8 | 8-10 | 8-12 | Tree Number | 8-6 | 8-8 | 8-10 | 8-12 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.810 | 0.837 | 0.864 | 0.830 | 7 | 0.865 | 0.839 | 0.840 | 0.806 |
| | .865 | .832 | .834 | .826 | | .860 | .808 | .849 | .818 |
| | .879 | .855 | .830 | .842 | | .850 | .842 | .830 | .812 |
| 2 | .856 | .835 | .822 | .805 | 8 | .840 | .854 | .837 | .822 |
| | .847 | .844 | .855 | .825 | | .846 | .818 | .818 | .821 |
| | .858 | .855 | .845 | .843 | | .828 | .853 | .861 | .825 |
| 3 | .850 | .793 | .832 | .812 | 9 | .839 | .850 | .829 | .807 |
| | .854 | .854 | .836 | .812 | | .837 | .836 | .849 | .808 |
| | .846 | .830 | .858 | .815 | | .866 | .832 | .829 | .819 |
| 4 | .842 | .846 | .885 | .812 | 10 | .832 | .846 | .855 | .811 |
| | .817 | .834 | .818 | .813 | | .819 | .841 | .819 | .816 |
| | .833 | .851 | .834 | .848 | | .854 | .851 | .824 | .819 |
| 5 | .877 | .841 | .817 | .827 | 11 | .874 | .834 | .829 | .817 |
| | .851 | .837 | .830 | .819 | | .840 | .828 | .820 | .812 |
| | .844 | .838 | .839 | .840 | | .855 | .841 | .827 | .799 |
| 6 | .833 | .816 | .816 | .832 | 12 | .837 | .835 | .833 | .818 |
| | .838 | .840 | .818 | .805 | | .864 | .795 | .860 | .817 |
| | .848 | .831 | .830 | .805 | | .855 | .833 | .812 | .812 |
| | | | | | Average | .847 | .836 | .835 | .818 |

Analysis of Variance

| Source of Variation | d.f. | Mean Square[b] | F |
|---|---|---|---|
| Total | 143 | | |
| Dates | 3 | 5.102 | 21.79[c] |
| Trees | 11 | 0.239 | 1.02 |
| Trees×Dates | 33 | 0.188 | 0.80 |
| Within Trees | 96 | 0.234 | |

[a] Each value represents 12 apples. Wayne County, New York, 1957.
[b] All values multiplied by 1,000 to eliminate zeros.
[c] Significant at 1% level.

**Table 4.—Specific gravities of McIntosh apples showing changes due to seasonal development.[a]**

| Sample | Date | Specific Gravity | Fruit in Sample | Trees in Sample | Sample | Date | Specific Gravity | Fruit in Sample | Trees in Sample |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *1956* | | | | | |
| 1 | 6-20 | 0.921 | 350 | 7 | 4 | 7-23 | 0.934 | 384 | 16 |
| | 6-24 | 0.998 | 350 | 7 | | 7-24 | 0.901 | 384 | 16 |
| | 6-26 | 0.967 | 350 | 7 | | 7-27 | 0.890 | 384 | 16 |
| (Harvest) | 9-26 | .877 | 84 | 7 | | 7-30 | 0.892 | 384 | 16 |
| 2 | 7- 2 | 0.945 | 512 | 16 | 5 | 7-31 | 0.890 | 264 | 11 |
| | 7- 3 | 0.925 | 512 | 16 | | 8- 1 | 0.880 | 264 | 11 |
| | 7- 5 | 0.927 | 512 | 16 | | 8- 3 | 0.879 | 264 | 11 |
| (Harvest) | 9-24 | 0.881 | 192 | 16 | | 8- 5 | 0.877 | 264 | 11 |
| | | | | | | 8- 8 | 0.875 | 264 | 11 |
| | | | | | (Harvest) | 9-23 | 0.862 | 132 | 11 |
| 3 | 7-16 | 0.931 | 512 | 16 | 6 | 8- 9 | 0.810[b] | 216 | 9 |
| | 7-17 | 0.948 | 512 | 16 | | 8- 9 | 0.866[b] | 216 | 9 |
| | 7-19 | 9.958 | 512 | 16 | | 8-11 | 0.890 | 216 | 9 |
| | 7-22 | 0.907 | 512 | 16 | | 8-13 | 0.832 | 216 | 9 |
| | 7-25 | 0.889 | 512 | 16 | | 8-17 | 0.865 | 216 | 9 |
| (Harvest) | 9-24 | 0.849 | 192 | 16 | (Harvest) | 9-23 | 0.878 | 108 | 9 |
| | | | | *1957* | | | | | |
| 1 | 7-16 | 0.878 | 420 | 20 | 2 | 7-18 | 0.902 | 546 | 26 |
| | 7-18 | 0.890 | 420 | 20 | | 7-25 | 0.841 | 546 | 26 |
| | 7-20 | 0.851 | 420 | 20 | (Harvest) | 9-11 | 0.818 | 200 | 20 |
| | 7-22 | 0.855 | 420 | 20 | | | | | |
| | 7-25 | 0.838 | 420 | 20 | | | | | |
| (Harvest) | 9-11 | 6.814 | 200 | 20 | | | | | |

[a] The values within each sample taken from the same trees. Each sample taken from a different group of trees. Wayne County, New York, 1956 and 1957.
[b] Rained 0.45 inches before dawn. First sample was taken early in the morning; the second sample was taken in late afternoon. Difference was not significant. Apples in samples 1 and 6 taken from an orchard which had a hardpan at 4 to 6 foot depth.

icantly underestimated the surface area computed by the planimeter method for Golden Delicious but not for McIntosh or for the total sample. The average difference between these methods was 1.54% (−0.738 cm.²) for McIntosh, 1.72% (+1.122 cm.²) for Golden Delicious and 0.36% (−0.270 cm.²) for all apples.

To determine the bias introduced when all the apples in a sample were assumed to be the same size, the total surface area for each sampling date and variety was computed using the volume displacement method and compared with the sum of the individual measurements. These results are given in table 1, column 3. The assumption that all apples in a sample were the same size produced a slight increase of surface area computed from the volume displaced, as can be readily demonstrated by plotting the volume against the surface area of a sphere and finding the sum of the surface areas of apples with volumes $v$ and $v'$, and for $2(v+v'/2)$ where v′ is appreciably larger than $v$. However, the size of this increase (bias) will depend upon the range of sizes of the apples; the greater the range in size, the greater the bias. In normal sampling it is doubtful that this bias would be greater than 1% of the total surface area inasmuch as the per cent bias computed from the values in table 1 ranged from 0.80 to 0.93% for McIntosh and from 0.51 to 1.11% for Golden Delicious.

*Weight Method.*—The use of weight to compute the surface area of an apple is an appealing method because of the ease and accuracy of determination. However, the assumption that all apples have the same specific gravity introduces an error varying with such conditions as variety, trees, location of fruit on the tree, the stage of development of the fruit at time of sampling, and seasonal conditions.

Variation in specific gravity owing to difference in variety does not appear important if the nonsignificant difference between McIntosh and Golden Delicious are representative. The variation between trees was relatively small and not significant in the 1958 McIntosh sample (table 2). Although the differences owing to location of the fruit sampled within a tree were of about the same magnitude as the difference between trees, these differences were consistent and significant at the 5% level (table 2 positions).

Variation caused by fruit development during the growing season and the variation from year to year appear to be the most important variables influencing specific gravity. Differences in specific gravity owing to development of the fruit (dates) were highly significant both in the 1958 experiment (table 2) and the 1957 McIntosh samples (table 3). In the latter case, highly significant differences were obtained for a 6-day development period.

The specific gravity also appears to vary greatly from season to season. At harvest time 1956, (table 4) the average specific gravity of McIntosh was 0.869; in 1957 (table 3) it was 0.816, and in 1958 (table 2) it was 0.804, in spite of the fact that sampling was earlier in each succeeding year. Thus the accuracy and precision of using a constant specific gravity would vary from year to year, within the season depending upon the development of the fruit, and within the tree depending upon the location from which the sample is taken.

The magnitude of the error introduced by assuming a constant specific gravity will vary according to the weight of the apple and the difference between the assumed and the actual specific gravity. An apple weighing 84.4 grams with a specific gravity of 0.802 would have a surface area of approximately 107.63 cm.². If it is assumed that this apple has a specific gravity of 0.902, then the surface area becomes 99.28 cm.², a difference of 8.53 cm.² or 7.8%. With an apple weighing 50 grams the difference becomes 7.5%. Generally, a difference between the actual and the assumed specific gravity of 0.1 or the maximum difference that might be expected to occur within a season (tables 2 and 4) will result in a 7% to 8% error in calculating the surface area. If a constant specific gravity of 0.833 is used for harvest data, this error will be considerably smaller as each 0.01 difference between the actual and assumed specific gravity will account for approximately 0.8% error and the maximum difference observed was 0.048 (table 4) or 3.8%. Generally the difference at harvest between the actual specific gravity and 0.833 will be less than the maximum observed and surface areas computed using $4.836\times\sqrt[3]{(\text{wt./}.833)^2}$ will be within 2% or 3% of the actual surface area.

The average difference between the weight using an assumed specific gravity of 0.833 and planimeter measurements (table 5) for all apples was 0.98% (+0.733 cm.²) of the planimeter value, with the surface areas computed by the planimeter significantly larger. When separated according to varieties the weight method significantly underestimated the planimeter surface area for Golden Delicious but not for McIntosh. The average difference was 0.35% (+0.287 cm.²) for McIntosh and 2.08% (+1.357 cm.²) for Golden Delicious.

*Comparison of Volume Displacement and Weight Methods.*—The standard deviation of the mean difference using the weight method was 0.537 cm.² for McIntosh and 0.462 cm.² for Golden Delicious, as compared with 0.463 cm.² and 0.354 cm.² for the volume displacement method. Thus, weight and the use of a constant specific gravity to calculate surface area are only slightly less accurate than the use of the volume displacement method based on comparisons with the planimeter standard. However, the deviation of the surface area computed from the volume displacement or the weight methods may be as large as 4% of the planimeter value.

**Table 5.—A statistical comparison of surface areas of McIntosh and Golden Delicious apples computed from planimeter measurements, volume displacement, and weight with an assumed specific gravity of 0.833.**

| | Square Centimeters | | | Difference as Per Cent of Planimeter |
|---|---|---|---|---|
| | $\bar{d}$ | $S_{\bar{d}}$ | "t" | |
| Planimeter—Volume displacement | | | | |
| McIntosh[a] | −0.738 | 0.463 | 1.595 | 1.54 |
| Golden Delicious[b] | +1.122 | 0.354 | 3.169** | 1.72 |
| All apples | −0.270 | 0.306 | 0.883 | 0.36 |
| Planimeter—Weight | | | | |
| McIntosh[a] | +0.287 | 0.537 | 0.534 | 0.35 |
| Golden Delicious[b] | +1.357 | 0.462 | 2.937* | 2.08 |
| All apples | +0.733 | 0.366 | 2.004* | 0.98 |

[a] Includes 1958 and 1959 data—56 degrees of freedom.
[b] 40 degrees of freedom.
* Significant at 5% level.
** Significant at 1% level.

*Other Methods.*—Huelin & Gallop (1951) reported a correction factor of 1.028 for correcting surface area computed from volume displacement measurements on Granny Smith apples. This figure compares quite favorably with the Golden Delicious results reported here, which would be 1.018, for the volume displacement method. The correction factor for McIntosh would be 0.985. The size of a correction factor will vary according to the shape of the apples. It will increase as the shapes vary from a sphere, thereby seriously limiting the use of such a factor no matter how accurately or precisely it is determined.

The surface area of apples computed from the average diameters as determined in this study greatly underestimated the true surface area (table 1). If diameter measurements have value in determining surface area, it would appear that the longitudinal diameter should consist of the maximum measurement from the calyx end to the stem end rather than the longitudinal measurement as used in this study.

Surface areas were also computed by use of several of Baten & Marshall's (1943) regression formulae. Their weight formula was the only one approaching the surface areas determined by planimeter measurement. This formula consistently overestimated the surface area (table 1).

Surface areas computed from the average of the diameters, and from Baten and Marshall's regression formulae for weight, were not compared statistically with the planimeter method because of the obvious significance of the differences.

*Sample Size.*—Using the formula for determining sample size needed to detect a given difference (Snedecor 1956, p. 485), it was found that a minimum sample of 23 McIntosh is required to determine surface area by volume displacement within 2% of the true mean ($\bar{x}=100$ cm.$^2$) at the 1% probability level. A minimum sample of 12 Golden Delicious would be sufficient for similar precision. The larger number of McIntosh required is probably due to the discrepancy observed in the August 15 sample. Larger numbers of either variety may be desirable.

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# Laboratory Studies on the Toxicity of Various Insecticides to Different Stages of the Oriental Fruit Moth[1]

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Abstract

Methods are described for testing large numbers of insecticides against adults, eggs, and newly hatched larvae of the oriental fruit moth (*Grapholitha molesta* (Busck)). Of 54 compounds tested by these methods, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate), parathion, methyl parathion, Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate), EPN, and Sevin® (1-naphthyl-*N*-methyl carbamate) were the most effective.

DDT was the first insecticide that gave economic control of the oriental fruit moth (*Grapholitha molesta* (Busck)). Since it became available for general use, many other insecticides have been tested against this insect for prevention of injury to ripe peach fruits. Early tests consisted almost entirely of applications to orchard plots to observe their effectiveness at harvest time several weeks or months later. Because of the time and labor involved, only a few materials could be studied each season. To increase the number that could be evaluated, methods were developed at Moorestown, New Jersey, for laboratory studies of the toxicity of various insecticides to adults, eggs, and newly hatched larvae. The results obtained provided a basis for the selection of materials for testing in orchard plots. The methods used and the results obtained in these studies from 1952 to 1957 are reported herein.

Chemical definitions of materials not bearing common names adopted by the Entomological Society of America appear at the end of this paper.

Methods.—The materials were tested as sprays only, usually at five concentrations, and were prepared from emulsifiable concentrates or wettable powders as formulated by the manufacturer. A few were formulated from technical compounds. One liter of spray was prepared in a percolator fitted with a short rubber tube and clamp. A 500-ml. aliquot withdrawn for experimental work was replaced with water, thereby reducing the spray concentration in the percolator by one-half for the next test. All sprays were agitated continuously with mechanical stirrers from the time of preparation to the completion of spray operations except during the short time the aliquots were being measured.

Sprays were applied at 30 pounds air pressure from a DeVilbiss nozzle that delivered 40 ml. per minute. The objects to be sprayed were placed on and over the middle of the turntable, which was operated at 10.5 r.p.m. Parathion formulated as a wettable powder was the standard used for comparison. The time during which sprays were applied in tests was the time required for 2 ounces of parathion per 100 gallons to wet peach leaves.

An attempt was made to expose the different stages of the oriental fruit moth to the test materials in a manner similar to their exposure under normal orchard conditions. The eggs are usually deposited on the leaves (Peterson & Haeussler 1930), and newly hatched larvae crawl over leaves and twigs to reach fruits or succulent twigs to feed. Most adults also are found on foliage and twigs.

Adults for the tests were produced in the laboratory on small green apples. The moths were sprayed in 16-mesh screen cages 6 inches in diameter and 8 inches long, the ends of which were tin-can lids. Fifty moths were sprayed in each test, and they were usually about 1 day old. Sprays were applied while the cages were revolving in both vertical and horizontal planes simultaneously. Moth mortality was determined 1 and 2 days later.

Eggs were obtained by placing pear branches bearing leaves with moths in small 16-mesh screen cages. Eggs numbering 100 or more were used in each test. Individual leaves bearing eggs were held for spraying on the turntable in a vertical position by placing the petiole in a clamp similar to a spring-type clothespin. Immediately after the spray was applied, the leaf petiole was placed in a small vial of water to maintain freshness until completion of observations. The toxicity of the insecticide was determined by examining the eggs with a microscope to ascertain the percentage that failed to hatch. Those not affected hatched in about 3 days.

The toxicity to newly hatched larvae was determined by inducing them to crawl over sprayed peach leaves and twigs. All but one of the leaves on a freshly cut twig were removed. With a cork cutter holes were made in two small green apples, one on top of the other, and the end of the twig was forced through these holes to 3 inches from the petiole. The twig was then cut 2 inches from the second apple applied and its end pushed through a rubber stopper, which was in turn inserted into a small bottle containing water. Paper toweling was wrapped around the apples to exclude spray. The assembly was then suspended on the turntable, with the leaf-bearing end of the twig downward. A small weight was attached to the end of the leaf to prevent its movement from this position. Sprayed assemblies were suspended on racks immediately below a 7-watt light to induce larvae to move rapidly upward toward the unsprayed apples in which the survivors were captured. The apples were about 1 inch in diameter and were picked at thinning time.

After the spray had dried, a pear leaf bearing eggs ready to hatch was attached with a paper clip to the sprayed peach leaf 3 inches from the twig. About 100 larvae were used on each leaf-twig assembly. The eggs hatched and surviving larvae usually entered the apples within 1 day. The apples were then placed in muslin-covered pint fruit jars containing strips of corrugated cardboard in which the mature larvae formed cocoons.

Some of the tests were made in a screened shed under normal spring and summer conditions, but most of them were made in chambers in which the relative humidity was about 60% and the temperature 80° F.

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[2] E. L. Plasket, W. E. Schmid, and David Norcross, biological aids, assisted in these studies.

**Table 1.—Per cent mortality of oriental fruit moth adults caused by insecticides at different spray concentrations.**

| Insecticide | No. of Replicates | Days After Application | Ounces per 100 Gallons | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.13 |
| Chipman R-6199 | 1 | 1 | 100 | 100 | 89 | 47 | 13 | 17 | — |
| | | 2 | — | — | 89 | 47 | 13 | 17 | — |
| Chlorthion | 8 | 1 | 100 | 97 | 64 | 45 | 29 | 14 | — |
| | | 2 | — | 100 | 98 | 92 | 82 | 62 | — |
| DDT | 3 | 1 | 52 | 40 | 10 | 3 | — | — | — |
| | | 2 | 95 | 78 | 32 | 12 | — | — | — |
| Diazinon | 2 | 1 | 100 | 100 | 97 | 86 | 92 | 50 | — |
| | | 2 | — | — | 100 | 94 | 92 | 74 | — |
| Dipterex | 3 | 1 | — | 100 | 100 | 83 | 34 | 4 | 0 |
| | | 2 | — | — | — | 96 | 75 | 22 | 16 |
| Dow ET-15 | 1 | 1 | — | — | 10 | 8 | 0 | 6 | 2 |
| | | 2 | — | — | 70 | 36 | 50 | 28 | 8 |
| EPN | 6 | 1 | — | 90 | 54 | 39 | 15 | 11 | — |
| | | 2 | — | 100 | 99 | 95 | 80 | 56 | — |
| Ethion | 2 | 1 | 78 | 69 | 33 | 14 | 0 | 0 | — |
| | | 2 | 100 | 100 | 96 | 44 | 15 | 18 | — |
| Guthion | 4 | 1 | — | — | 74 | 55 | 44 | 17 | 3 |
| | | 2 | — | — | 91 | 82 | 61 | 46 | 21 |
| Hercules AC-3895 | 1 | 1 | 100 | 100 | 100 | 100 | 88 | 71 | — |
| Methoxychlor | 2 | 1 | 20 | 15 | 0 | 0 | — | — | — |
| Methyl parathion | 3 | 1 | — | — | 100 | 97 | 87 | 87 | 56 |
| | | 2 | — | — | — | 100 | 98 | 99 | 96 |
| Parathion | 16 | 1 | — | — | 96 | 90 | 79 | 69 | 61 |
| | | 2 | — | — | 100 | 98 | 94 | 92 | 88 |
| Perthane | 1 | 1 | 100 | 76 | 56 | 9 | — | — | — |
| | | 2 | — | 89 | 76 | 9 | — | — | — |
| Pirazinon | 2 | 1 | — | 100 | 100 | 100 | 100 | — | — |
| Ronnel | 1 | 1 | — | — | 44 | 40 | 27 | 24 | 0 |
| | | 2 | — | — | 94 | 80 | 66 | 60 | 44 |
| Ryania | 1 | 1 | 0 | 0 | — | — | — | — | — |
| | | 2 | 0 | 0 | — | — | — | — | — |
| Sevin | 2 | 1 | 100 | 100 | 100 | 100 | 93 | 84 | — |
| Tedion | 1 | 1 | 2 | 9 | 0 | 0 | — | — | |
| | | 2 | 33 | 44 | 0 | 0 | — | — | — |
| Thiodan | 2 | 1 | 73 | 47 | 8 | 0 | — | — | — |
| | | 2 | 74 | 50 | 27 | 3 | — | — | — |

RESULTS.—Adult mortality caused by various concentrations of insecticides is shown in table 1, egg mortality in table 2, and larval mortality in table 3. Mortalities caused by one concentration of selected materials against all these stages are shown in table 4. Adult mortality in check observations averaged 2% during the first day and 11% during the second day in 68 tests; egg mortality averaged 5% in 197 tests, and survival of larvae averaged 56% in 97 tests.

Guthion,® parathion, methyl parathion, Chlorthion,® EPN, and Sevin® were more effective against all stages than the other materials tested, and ryania, Tedion,® Perthane,® and methoxychlor were the least effective. Most of the materials were more toxic to adults than to the other stages. However, methoxychlor was comparatively low in toxicity to adults, and ryania caused no mortality even when applied at dosages up to 64 ounces per 100 gallons. Mortality among adults sprayed in cages with 0.25 to 2 ounces of parathion, EPN, and Chlorthion and 1 to 8 ounces of DDT per 100 gallons averaged 13% greater than among adults placed in such cages immediately after the sprays had dried. Some of the materials

**Table 2.—Per cent mortality of oriental fruit moth eggs caused by insecticides at different spray concentrations.**

| Insecticide | No. of Replicates | Ounces per 100 Gallons | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.13 |
| Bayer 24869 | 2 | — | 16 | 11 | 17 | 6 | 6 | — |
| Bayer 25141 | 2 | — | 93 | 78 | 46 | 32 | 30 | — |
| Chipman R-6199 | 4 | 60 | 43 | 34 | 20 | 10 | 5 | — |
| Chlorthion | 14 | — | 100 | 88 | 71 | 56 | 41 | 24 |
| DDT | 1 | 19 | 21 | 15 | 4 | 11 | — | — |
| Diazinon | 2 | — | 30 | 29 | 21 | 16 | 13 | 6 |
| Demeton | 1 | 43 | 57 | 51 | — | — | — | — |
| Dipterex | 6 | 40 | 32 | 19 | 12 | 6 | — | — |
| Dow ET-15 | 4 | — | — | 16 | 7 | 7 | 5 | — |
| EPN | 8 | — | 74 | 64 | 54 | 52 | 49 | — |
| Ethion | 4 | 10 | 4 | 2 | 5 | 5 | 3 | — |
| Guthion | 15 | — | — | 90 | 87 | 81 | 72 | 44 |
| Delnav | 2 | 2 | 5 | 3 | 2 | 3 | — | — |
| Hercules 2118 | 1 | 7 | 10 | 4 | — | — | — | — |
| Hercules 1986 | 1 | 17 | — | 5 | — | — | — | — |
| Hercules 2032 | 1 | 31 | 27 | 39 | — | — | — | — |
| Hercules 3273 | 1 | 71 | 73 | 45 | — | — | — | — |
| Hercules AC 3895 | 4 | 58 | 44 | 22 | 17 | — | — | — |
| Malathion | 3 | 95 | 73 | 60 | 44 | 30 | — | — |
| Methoxychlor | 1 | — | — | 12 | 15 | 19 | 19 | — |
| Methyl parathion | 20 | — | 99 | 99 | 97 | 96 | 91 | 75 |
| Parathion | 24 | — | — | 93 | 91 | 76 | 61 | 41 |
| Perthane | 4 | 5 | 6 | 2 | 3 | — | — | — |
| Phorate | 1 | 56 | 48 | 42 | — | — | — | — |
| Phosdrin | 1 | 97 | 94 | 80 | — | — | — | — |
| Pirazinon | 1 | — | — | 89 | 86 | 78 | 43 | 38 |
| Ronnel | 2 | — | — | 2 | 1 | 5 | 3 | — |
| Ryania | 1 | 5 | 8 | — | — | — | — | — |
| Sevin | 8 | 100 | 92 | 75 | 43 | 30 | 19 | — |
| Tedion | 4 | 4 | 5 | 3 | 4 | 4 | — | — |
| Thiodan | 3 | 3 | 3 | 7 | 3 | — | — | — |
| Trithion | 3 | 25 | 15 | 8 | 6 | 4 | — | — |

varied considerably in their toxicity to different stages. Dow ET-15, Thiodan,® and ethion were fairly effective against adults but markedly less effective against eggs. Phosdrin,® Pirazinon,® and phorate were fairly effective against eggs but not against larvae.

The application of some insecticides to adults appeared to stimulate egg deposition. This characteristic was particularly apparent when the toxic action of materials was retarded by cool weather. For example, on an average 97 eggs were deposited by lots of 25 females sprayed with EPN at 0.07 to 1 ounce per 100 gallons and 17 eggs by untreated females during 2 days after sprays were applied. This tendency was observed also with low concentrations of parathion. Practically all the organophosphorus compounds quickly affect the ability of adults to fly and adhere to plant surfaces. It is not likely, therefore, that stimulation of egg deposition would appreciably decrease their effectiveness when applied to orchards, as adults only slightly affected become active and in their effort to escape probably would not remain long in trees to deposit eggs.

**Table 3.—Per cent mortality of newly hatched larvae of the oriental fruit moth that crawled over leaves and twigs sprayed with insecticides at different concentrations.**

| Insecticide | No. of Replicates | Ounces per 100 Gallons | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.13 |
| Bayer 24869 | 2 | — | 21 | 17 | 24 | 0 | 4 | — |
| Bayer 25141 | 2 | — | 69 | 29 | 30 | 10 | 3 | — |
| Chipman R-6199 | 2 | 49 | 47 | 16 | 14 | 21 | 0 | — |
| Chlorthion | 7 | 100 | 99 | 82 | 63 | 50 | 22 | 19 |
| DDT | 2 | 86 | 44 | 25 | 13 | 0 | — | — |
| Demeton | 1 | 16 | 38 | 6 | — | — | — | — |
| Diazinon | 4 | 85 | 45 | 28 | 29 | 30 | — | — |
| Dipterex | 4 | 65 | 53 | 33 | 12 | 1 | 0 | — |
| Dow ET-15 | 2 | — | — | 28 | 8 | 14 | 18 | 6 |
| EPN | 4 | — | 100 | 99 | 98 | 93 | 64 | 37 |
| Ethion | 2 | 40 | 26 | 23 | 27 | 11 | 12 | — |
| Guthion | 8 | — | — | 81 | 52 | 45 | 31 | 16 |
| Delnav | 1 | — | 23 | 0 | 0 | — | — | — |
| Hercules 1986 | 1 | 58 | 68 | 0 | — | — | — | — |
| Hercules 2032 | 1 | 30 | 53 | 39 | — | — | — | — |
| Hercules 2118 | 1 | 31 | 43 | 58 | — | — | — | — |
| Hercules 3273 | 1 | 10 | 10 | 0 | — | — | — | — |
| Hercules AC 3895 | 2 | 65 | 41 | 13 | 9 | 6 | — | — |
| Malathion | 2 | 99 | 94 | 63 | 54 | 49 | — | — |
| Methoxychlor | 1 | 70 | 33 | 23 | 20 | 14 | — | — |
| Methyl parathion | 3 | — | 84 | 78 | 62 | 57 | 30 | — |
| Parathion | 11 | — | 100 | 91 | 81 | 58 | 47 | 19 |
| Perthane | 2 | 20 | 3 | 6 | 5 | — | — | — |
| Phorate | 1 | 0 | 0 | 0 | — | — | — | — |
| Phosdrin | 1 | 0 | 0 | 0 | — | — | — | — |
| Pirazinon | 1 | — | 30 | 17 | 6 | 0 | — | — |
| Ronnel | 1 | — | — | 11 | — | 20 | 3 | 0 |
| Ryania | 1 | 7 | 28 | 11 | — | — | — | — |
| Sevin | 4 | 95 | 84 | 58 | 24 | 22 | 1 | — |
| Tedion | 2 | 12 | 25 | 4 | 9 | 0 | 0 | — |
| Thiodan | 2 | 24 | 12 | 22 | 18 | 6 | — | — |
| Trithion | 1 | 89 | 36 | — | — | 14 | — | — |

**Table 4.—Per cent mortality of adults, eggs, and larvae of the oriental fruit moth caused by the application of one spray concentration of various insecticides.**

| Insecticide | Ounces per 100 Gallons | Adults | Eggs | Larvae |
|---|---|---|---|---|
| Acetate of 2,4-dinitro-6-phenyl phenol | 16 | 84 | 8 | 0 |
| Aldrin | 8 | — | 1 | 14 |
| Dieldrin | 4 | — | 4 | 14 |
| Dilan | 8 | 98 | 8 | 11 |
| Endrin | 4 | 100 | 4 | 0 |
| Heptachlor | 16 | 65 | 17 | 11 |
| Isodrin | 8 | — | 4 | 0 |
| *O,O*-diethyl-*O*-(*p*-methoxybenzyl) thiophosphate | 16 | 100 | 8 | 2 |
| *O,O*-diethyl-*O*-cinnamyl thiophosphate | 16 | 100 | 4 | 41 |
| *O,O*-diethyl-*O*-(o-veratryl)thiophosphate | 16 | 100 | 11 | 22 |
| *O,O*-diethyl-*O*-benzhydryl thiophosphate | 16 | 48 | 2 | 2 |
| *O,O*-diethyl-*O*-hydrocinnamyl thiophosphate | 16 | 96 | 8 | 0 |
| *O,O*-diethyl-*O*-(α-propyl benzyl) thiophosphate | 16 | 100 | 15 | 55 |
| *O,O*-diethyl-*O*-(α-propyl-p-methoxybenzyl) thiophosphate | 16 | 100 | 11 | 0 |
| *O,O*-diethyl-*O*-(α-phenyl propyl)piperonyl thiophosphate | 16 | 94 | 15 | 28 |
| *O,O*-diethyl-*O*-(4-chloro-propylbenzyl) thiophosphate | 16 | 100 | 2 | 22 |
| Pentachlorophenyl propionate | 16 | 98 | 5 | 35 |
| Propyl thiopyrophosphate (NPD) | 4 | 100 | 5 | 31 |
| Strobane | 16 | 79 | 11 | 19 |

None of the materials seemed to arrest embryo development. Affected eggs produced apparently fully formed larvae, which died within the chorion, usually without attempting to emerge, although a few died with their heads protruding through the chorion. Chlorthion and parathion were consistently more toxic to eggs ready to hatch than to newly deposited eggs. The effect of parathion on eggs of different ages was similar to that observed by Smith (1955) on eggs of the peach tree borer (*Sanninoidea exitiosa* (Say)). Guthion was toxic to eggs of both ages, but slightly more so to newly deposited eggs. The mortality of eggs of different ages caused by these insecticides is shown in table 5.

Observations on newly hatched larvae showed no sexual difference in susceptibility to concentrations of parathion,

**Table 5.—Per cent mortality of oriental fruit moth eggs of different ages exposed to Chlorthion, parathion, and Guthion.**

| Insecticide | Age of Eggs (Days) | No. of Replicates | Ounces per 100 Gallons | | | |
|---|---|---|---|---|---|---|
| | | | 2 | 1 | 0.5 | 0.25 |
| Chlorthion | 0–1 | 7 | 79 | 49 | 19 | 18 |
| | 2–3 | 7 | 95 | 94 | 94 | 65 |
| Parathion | 0–1 | 6 | 91 | 87 | 66 | 38 |
| | 2–3 | 10 | 94 | 92 | 83 | 76 |
| Guthion | 0–1 | 5 | 92 | 89 | 87 | 77 |
| | 2–3 | 6 | 82 | 78 | 69 | 61 |

methyl parathion, and EPN ranging from 0.04 to 2 ounces per 100 gallons. In 14 tests of these materials the adults (29 per test, average) from larvae that survived averaged 34% females and 33% in check observations.

Orchard plot tests were conducted in the vicinity of Moorestown each year that laboratory studies were made. The toxicity of some of the insecticides that compared favorably with parathion (standard) in the laboratory were considerably less effective in the orchard. None of them was more effective, comparatively, in the orchard than in the laboratory.

Laboratory tests proved to be a valuable adjunct to orchard tests. They indicated the comparative toxicity of materials immediately after application, the materials that were worthy of tests in orchard plots, the spray concentration that should be used in initial orchard tests, and the need to time orchard application of certain materials to coincide with maximum abundance of the stage against which they were most effective.

### CHEMICAL NAMES OF INSECTICIDES TESTED

Bayer 24,869—1-chloro-2-propyl 2,2-dichlorovinyl ethyl phosphate

Bayer 25,141—*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate

Chipman R-6199—*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]

Chlorthion—*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate

Diazinon®—*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

Dilan—1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane

Dipterex®—*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate

Dow ET-15—*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramidothioate

Guthion—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl-methyl)phosphorodithioate

Delnav®—2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)

Hercules 1986—*S*-(*O,O*-diethyl phosphorodithioate) of trichlorotetrahydropyranthiol

Hercules 2032—*S*-(*O,O*-diethyl phosphorodithioate) of *p*-oxathiane-3-thiol.

Hercules 2118—*S-S*-bis(*O,O*-dimethyl phosphorodithioate) of 2,3-*p*-dioxanedithiol

Hercules 3273—*S*-(*O,O*-dimethyl phosphorodithioate) of *p*-2-oxathiene-X-thiol

Hercules AC 3895—2,2-bis(ethylthio)vinyl diethylphosphate

Perthane-1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane

Phosdrin—1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical

Pirazinon—*O,O*-diethyl *O*-3-(2-propyl-6-methyl-4-pyrimidinyl) phosphorothioate

Sevin—1-naphthyl *N*-methyl carbamate

Strobane®—A terpene polychlorinate with a chlorine content of approximately 66%

Tedion—2,4,4,5-tetrachlorodiphenyl sulfone, 80% technical

Thiodan—6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide

Trithion®—*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate

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## INDEX XVI

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# SCIENTIFIC NOTES

## Starvation Tests with Larvae of the Cinnabar Moth[1]

HARRY L. PARKER, *Entomology Research Division, Agr. Res. Serv. U.S.D.A.*

As a preliminary measure to determine whether the cinnabar moth (*Tyria jacobaeae* (L.)) might safely be introduced into the United States for possible biological control of tansy ragwort (*Senecio jacobaea* L.), tests were conducted in France to learn whether the moth could complete its development on certain plants other than ragwort. Although results of starvation tests cannot be accepted as a final measure of an insect's host-plant range, they do constitute an essential precautionary step to be taken prior to the movement of a phytophagous insect from one country to another for attempted control of a plant.

The cinnabar moth was recognized by Cameron (1935) as one of the principal insect enemies of tansy ragwort. In studies prior to its introduction into New Zealand from England, he determined that larvae could not survive on lettuce, chrysanthemum, sunflower, goldenrod, aster, Michaelmas-daisy, and dahlia. In order to supplement Cameron's observations and thus provide added assurance that the cinnabar moth will not be a threat to useful plants in the United States, additional starvation tests were undertaken at the Entomology Research Division's European Parasite Laboratory, Nanterre, Seine, France, on the following plants: artichoke (*Cinara scolymus* L.), *Aster* sp., *Cosmos* sp., daisy (*Leucanthemum vulgare* L.), honeysuckle (*Lonicera* sp.), hops (*Humulus lupulus* L.), marigold (*Calendula* sp.), safflower (*Carthamus tinctorius* L.), strawberry (*Fragaria chiloensis* Duch.) and *Zinnia* sp.

Some of the tests were performed with first-stage larvae hatching from eggs laid in the laboratory and some with larvae collected on tansy ragwort in the field.

Larvae together with leaves and stems of the test plant were placed in a Sôme dish, which is a cylindrical glass container, half filled with water and covered by a cloth tightly stretched across its top. The larvae and food were placed on this cloth, and a smaller glass dish was inverted over them. This arrangement permits humidity to be varied by addition of different quantities of salt to water and allows free circulation of air. The plant food thus remains fresh for several days, and the larvae do not suffer from drought. The dishes were examined daily and the food changed usually every day, but sometimes every other day. Notes were made on the feeding, defecation, molting, pupation, and death. Dissections were made to determine the food material in intestine.

At the time of larval hatching in 1957, *Cosmos*, hops, safflower and *Zinnia* were not available for testing; consequently tests on these plants were conducted later with larvae from ragwort plants taken in the field. Later tests were also made on safflower with first-stage larvae.

ARTICHOKE (heads).—Two tests with a total of 201 first-stage larvae showed a small amount of feeding at bases of bracts, especially along cut edges. No larvae molted into second stage; all died by seventh day.

ASTER.—Three tests with a total of 310 first-stage larvae showed slight feeding along cut edges of leaves, leaf nerves, and bases of stems. No larvae molted to the second stage or lived longer than 4 days.

COSMOS.—Twenty-seven second- and 13 third-stage field-collected larvae fed rather freely on the stems and leaf veins, particularly after 3 days of fasting. Seventeen died after 5 days, several molted into the next stage, one into the fifth stage, and the last three died on 20th day. Evidently the larvae had considerable initial impetus from field feeding by the younger stages on the ragwort. *Cosmos* apparently does not furnish elements necessary for completion of the life cycle.

DAISY.—Ninety-six newly hatched larvae did not feed, and all died within 4 days. In a second test, with 130 newly hatched larvae, one-half died in the first stage by the third day. A small amount of feeding was evident on cut edges of leaves and veins. By the sixth day only six larvae were still living. Three lived until the 14th day and molted to the second stage. The last larva died on the 20th day, and none molted to the third stage.

HONEYSUCKLE.—Thirty-one field-collected larvae in the third and fourth stages were placed with leaves, flowers, and fruit. No feeding whatever had occurred by the third day. On the fifth day 19 were dead and several had molted, and about 2 sq. mm. was eaten on the cut edge of a leaf. The remaining larvae died by the eighth day without further feeding.

In a repeat test 19 second- and 39 third-stage field-collected larvae were used. By the fifth day small areas about the diameter of a pencil lead had appeared eaten along the cut leaf margins. On the seventh day, 4 sq. mm. on an edge of a leaf was eaten; all larvae were dead or dying.

(Tests were conducted on this plant because an assistant had once observed this caterpillar on honeysuckle.)

HOPS.—Forty field-collected larvae of the second and third stages died within 6 days without attempting to feed.

MARIGOLD.—Same as for hops.

SAFFLOWER (1957 tests).—Forty field-collected larvae in the late second and early third stages were used in the first test. By the fifth day some were feeding on the bases of the leaf ribs and cut stems; by the seventh day 19 were dead. Stem feeding was considerable, and one leaf showed an eaten spot. By the eighth day nine more were dead. About one-third of each of the two leaves was consumed, and three stems were hollowed out and eaten off. By the 12th day only three larvae were left alive and they died on the 13th day.

In a repeat test 19 field-collected third- and fourth-stage larvae did not feed until the fourth day. On the fifth day there was considerable feeding on leaves and stems of plants, which were chewed up, hollowed out, and the pieces scattered around the dish, which was fouled by liquid fecal matter. By the eighth day only 10 larvae were alive; some molted, 1 pupated on ninth day, and 2 on 13th day. Although more feeding, molting, and pupating occurred than on any other plant, it is probable that these larvae gained sufficient impetus from feeding in younger stages on ragwort to enable them to complete their development.

SAFFLOWER (1958 tests).—Fifty-one newly hatched larvae were placed on fresh leaves over water. By the fourth day most of them were dead, but two holes, each about the size of a pinhead, were eaten in the leaves. On the sixth day there were additional feeding areas about the size of fly specks, and on the seventh day one spot about 1 mm. in diameter was eaten. At this time two wandering larvae remained. One of these died on the 10th day. Additional small spots were eaten at the base of veins and cut stems. The last larva died on the 12th day.

In a repeat test 28 newly hatched larvae were placed on fresh leaves over water. By the third day 20 were dead and two were feeding on the fringe at the base of a leaf. On the fourth day, the cut edge of the leaf showed evidence of feeding and the dish contained 40 to 50 tiny fecal pellets. On the seventh day additional pinpoint feeding on cut-leaf margins was evident, and five larvae were turning pink-orange. On the eighth day one molted to the second stage, but did not feed. On the ninth day the second molted. One died on the 11th and the other on the 12th day without further feeding.

In another test 117 eggs about to hatch were placed in a cage in a potted plant. By the fifth day 16 dead larvae and five unhatched eggs were removed. On the 17th day no live larvae could be found, and there was no sign of any feeding.

[1] Accepted for publication December 7, 1959.

STRAWBERRY.—About 50 half-grown field-collected larvae were confined with leaves. No feeding whatever. (This test was made several years ago following a report that a strawberry patch was being devastated by a caterpillar. The writer visited this patch and found larvae of this moth crawling all over the plants. Closer examination showed plenty of small plants of *Senecio vulgaris* on which the larvae were feeding. The strawberry plants were not attacked in the slightest degree.)

ZINNIA.—Twenty-five field-collected larvae, 11 in the fourth and 14 in the fifth stage were placed on fresh leaves and stems. By the sixth day there was some feeding on a thick central leaf vein and four larvae had pupated. By the seventh day only three larvae were still living, and one of them pupated on the eighth day. A small amount of feeding on one leaf was noted on the ninth day. The last larva died on the 14th day. As was evident from the safflower tests it appeared that last stage larvae could complete their development and pupate as a result of reserves available from early feeding on ragwort.

CONCLUSION.—From these tests it does not seem that the cinnabar moth could complete its life cycle from egg to adult on any of the plants with which it was placed.

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## Greenbug Dispersal and Distribution of Barley Yellow Dwarf Virus in Wisconsin[1]

JOHN T. MEDLER and PHILIP W. SMITH[2]

On June 4, 1959, the writers received telephone calls from several farmers and county agents who were concerned with an unusual abundance of aphids in oat fields Surveys conducted June 4 and 5 revealed heavy infestations of the greenbug, *Toxoptera graminum* (Rond.), near New Glarus, Green County, and south of Galesville, Trempeleau County. Subsequent surveys showed a general distribution of the greenbug in most of the State. By June 15, a few additional localized heavy infestations had been detected (fig. 1). According to a questionnaire that was circulated among county agents, 18 of Wisconsin's 71 counties had aphids sufficiently abundant to stimulate insecticide treatment of about 20,000 acres.

Although the greenbug does not overwinter in Wisconsin, specimens are occasionally found during the summer on grasses and small grains. The appearance of the species in the Northern States is dependent upon wind dispersal from areas of normal distribution in the South. During an outbreak unusual ecological conditions must exist, first in the South to produce an abundant reservoir of migrants, and second in the North to allow for a population buildup of the migrants after their arrival. Outbreaks occur in Oklahoma about once every 4 years, according to Fenton & Dahms (1951). Outbreaks in the northern United States occur unexpectedly, such as in Minnesota in 1926 (Wadley 1931) and in Wisconsin in 1959.

The initial dispersal of greenbugs into Wisconsin took place during May 2 to 6 in association with Statewide strong southerly winds and maximum daily temperatures above 90° F. The dispersal into Wisconsin was only a part of a widespread migration that occurred in the Middle West. According to data obtained from yellow pan traps placed in small grain plots at Columbia, Missouri, Manhattan, Kansas, and Madison, Wisconsin (table 1), an increased activity of alate greenbugs occurred during the period April 27 to 30 at Columbia, April 27 to May 7 at Manhattan, and May 3 to 6 at Madison. The migrant aphids that arrived in Wisconsin no doubt became generally established, because many oat fields were in an early stage of growth because of later than normal planting dates caused by unusually cold and wet weather in April. Weather and host conditions were favorable for greenbugs in May and June, and wind trap records at Madison showed a continuous high level of air-borne greenbugs. During early June, these air-borne aphids increased populations in oat fields. Damage was associated with ecological conditions favoring the attraction and accumulation of aphids The greenbugs were more abundant in wind-protected places, on lighter soils, along field margins where plants were widely spaced and of unthrifty growth, and where fields had been planted late. Toward the last of June, locally heavy showers, advance in grain

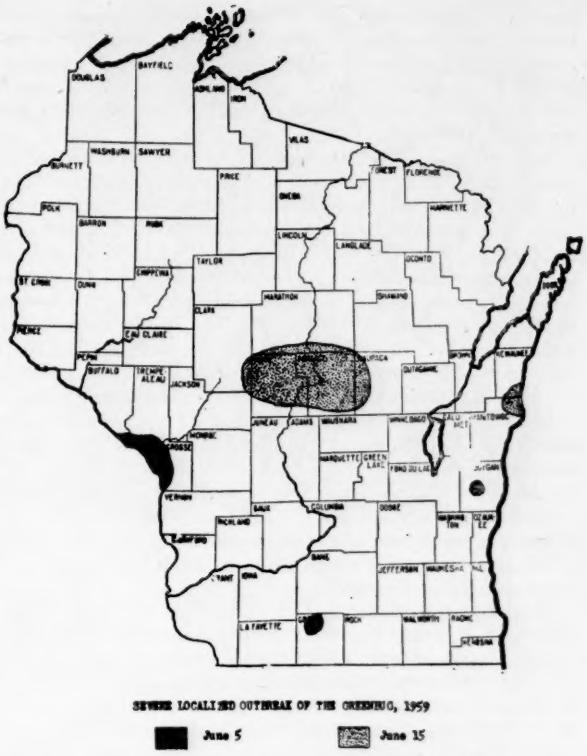


FIG. 1.—Distribution of heavy infestations of the greenbug in Wisconsin, June 1959.

**Table 1.—Numbers with dates, of greenbugs collected in traps[a] at three locations, 1959.**

| COLUMBIA, MO.[b] | | MANHATTAN, KANS.[c] | | MADISON, WIS.[d] | |
|---|---|---|---|---|---|
| Date | No. | Date | No. | Date | No. |
| April 2– 6 | 1 | April 9–24 | 0 | April 12–31 | 0 |
| 6–20 | 0 | 24–26 | 2 | May 1– 3 | 1 |
| 20–25 | 2 | 26–29 | 4 | 3– 6 | 7 |
| 25–27 | 1 | 29–May 1 | 17 | 6–12 | 5 |
| 27–30 | 12 | May 1– 3 | 3 | 12–14 | 0 |
| | | 3– 7 | 17 | | |
| | | 7–12 | 2 | | |
| | | 12–13 | 5 | | |
| | | 13–14 | 1 | | |

[a] Two canary-yellow pans, 30×30×10 cm., were placed at the margins of grain fields at each location. Aphids were trapped by water in the pans.
[b] Collected by D. C. Peters.
[c] Collected under direction of H. C. Knutson.
[d] Collected by G. B. Orlob.

[1] Accepted for publication January 11, 1960.
[2] Associate Professor, University of Wisconsin and Survey Entomologist, Wisconsin Department of Agriculture, respectively. The authors express appreciation to Miss L. M. Russell, Entomology Research Division, U.S.D.A. for confirming the identification of the species.

growth, and predaceous insects[3] reduced the greenbug populations, especially in southern parts of the State. Reports and observations on greenbug damage dropped markedly. By the end of June it was apparent that the greenbug outbreak was localized and the severely damaged acreage was limited. Very few fields with dense stands were affected.

It is well known that greenbug injury is much more serious than expected in proportion to numbers of aphids. Even light infestations may kill young plants and tillers. The characteristic severe injury results from both sap removal and injection of a toxic salivary secretion. Furthermore, the greenbug is capable of transmitting virus diseases, including barley yellow dwarf (Oswald & Houston 1953). The yellow dwarf virus in oats is known as red leaf. The symptoms of barley yellow dwarf virus in barley and oats can be confused with the symptoms of greenbug feeding. When both virus and feeding damage occur in young plants, the yield of grain is considerably reduced or lost entirely.

In 1959, red leaf was more prevalent in Wisconsin than for nearly a decade. Symptoms were noted first at Madison May 23 and were observed in many oat fields in early June. By the middle of June the disease was being reported commonly. The increased incidence of red leaf probably can be attributed to the widespread occurrence of the greenbug vector. However, some of the virus was undoubtedly spread by other vector species of grain aphids found commonly in 1959.

It should be mentioned that unthrifty fields of oats had symptoms that variously might be attributed to greenbug feeding, red leaf, haloblight, residual herbicide injury, and drought. In some heavily infested fields treatments with malathion or parathion were made in early June, effectively reducing the greenbugs. However, a widespread infection of virus had already taken place that caused serious injury after the greenbugs were controlled. Medler & Shands (1958 unpublished) summarized 5 years of insecticide tests to control the aphid vectors of yellow dwarf virus, and concluded that the virus could not be completely eliminated from plots by controlling aphids. However, a major benefit of insecticide treatment was prevention of aphid buildup in plots and secondary spread of virus.

The conclusion may be reached that the 1959 greenbug problem in Wisconsin was associated with later-than-normal plantings of grain, early appearance of a heavy migration, and subsequent weather that favored a heavy buildup of aphid population in localized environments.

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[3] The following coccinellids were identified by M. S. Conrad, Entomologist, Wisconsin Department of Agriculture: *Brachyacantha ursina* (Fab.), *Coccinella novemnotata* Hbst., *Coleomegilla maculata* (DeG.), *Hippodamia convergens* G.-M., *H. parenthesis* Say, *H. tredecimpunctata* L.

## Currant Fruit Weevil, *Pseudanthonomus validus*, a Pest of Cultivated Highbush Blueberry[1]

William E. Tomlinson, Jr.[2]

Weevils reared from grubs infesting cultivated highbush blueberry fruit at Sandwich, Massachusetts, in the spring of 1953 were identified by the Insect Identification Laboratories, U. S. Department of Agriculture, as the currant fruit weevil, *Pseudanthonomus validus* Dietz. Since that time this weevil has been found in all blueberry fields examined in southeastern Massachusetts. Most infestations have been light, with only an occasional berry infested. However, in 1958 the crop was reduced considerably by this weevil in the field in which they were first found in 1953.

Fig. 1.—Usual oviposition site of currant fruit weevil in cultivated blueberry (egg pushed from stem by growth of stem as in this photo is rarely encountered).

The currant fruit weevil was reported to be a pest of wild lowbush blueberries in Maine by Woods (1915), and in Nova Scotia by Maxwell & Pickett (1949). Cooley (1914) reported it as a pest of currants in Montana, which accounts for its common name. Blatchley & Leng (1916) gave the distribution as Ohio, Indiana, Canada, Nebraska, Colorado, and New Mexico. There is no record in the literature of its infesting cultivated highbush blueberries, or of its occurrence in Massachusetts.

Woods described the life history and habits of the species in Maine blueberry fields. Because the season is earlier in Massachusetts than in Maine, the activities occur 10 days to 2 weeks earlier in the former, with greatest beetle activity and egg laying occurring on warm days in late May and early June.

Egg laying habits in cultivated highbush blueberry differ slightly from observations made by Woods on wild lowbush blueberry, who found that the female usually oviposits in the calyx

[1] Miscellaneous Contribution No. 1014 of the University of Massachusetts College of Agriculture Experiment Station, Amherst, Massachusetts. Accepted for publication January 8, 1960.

[2] Professor, Cranberry Experiment Station, Department of Entomology and Plant Pathology.

lobes but occasionally deposits an egg just beneath the skin at the stem end of the fruit. In cultivated highbush blueberries, oviposition at the stem end or in the stem of the berry itself was usual (fig. 1), while eggs in the calyx lobes were rarely encountered. When the egg is laid in the stem, the larva on hatching tunnels up the stem into the berry to finish feeding.

Excellent control of the currant fruit weevil was obtained in 1959 with an application of 3 ounces of actual dieldrin per acre, applied from aircraft just before the first blossoms of early blooming varieties opened.

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## Control of the Tropical Rat Mite[1]

WALTER EBELING, *University of California, Los Angeles*

The tropical rat mite, *Ornithonyssus bacoti* (Hirst) (= *Bdellonyssus*) is a gray to pale yellowish gray mite, changing to red or black when engorged with blood. It commonly occurs on rats throughout the world, particularly in tropical and subtropical regions, but also in some Temperate Zone areas. Skaliy & Hayes (1949) found the female mites to vary in length from an average of 1.15 mm. for unfed adults to 1.41 mm. for engorged adults. The males were about two-thirds the length of the females. The average length of life of adult females was 61.9 days, the average number of eggs laid was 98.8, and the life cycle, from egg to egg, was 10 to 12 days.

When rats occur in houses their fecal pellets may be found in the attic and can usually be seen from the crawl hole. When rats are killed, the mites leave their bodies and may travel great distances, particularly along the heating pipes in the walls, for when they are not engorged with blood they are very active.

When the mites are abundant, they may be found anywhere in the home. Both nymphs and adults may attack man. Their bites may produce irritation and sometimes painful dermatitis that may continue for 2 or 3 days, leaving red spots (maculae) on the infested parts of the body (fig. 1). Scratching may result in secondary infections. In some families some individuals are affected and others are not. Sometimes much time and money are spent on ineffective medication and usually it has been difficult for the infested person to obtain a correct diagnosis. This acariasis cannot be distinguished from flea bites and is sometimes misidentified as scabies.

CONTROL.—The complete control of rats would, of course eventually result in the elimination of rat mites from the infested premises. However, rat control often proves to be difficult and "rat proofing" of an attic may also prove to be difficult and sometimes very expensive. It should also be borne in mind, as stated previously, that trapping or otherwise killing rats may for a time increase the attacks on the inhabitants of the house because of the mites' leaving the bodies of the dead rats.

Many insecticides and acaricides have been used for control (Mallis 1954, Baker *et al.* 1956). All insecticides depending on toxic action have the serious limitation of losing their toxicity too rapidly, particularly at the high summer temperatures of the attic. Reinfestation may then occur. HCN gas fumigation has been used successfully, but it is expensive and leaves no residue.

The successful use of a fluoridated silica aerogel dust, blown into attics for the prevention of drywood termites (*Kalotermes minor* (Hagen)) (Ebeling & Wagner, 1959), suggested a similar use of this material for the control of the tropical rat mite. In five infested houses and one two-story apartment house, in each of which one or more inhabitants had been attacked by rat mites for prolonged periods, a silica aerogel containing 4.7% of ammonium fluosilicate[2] was blown into the attic at the rate of 1 pound to 1,000 sq. ft. of attic area. For four of the houses, the dust was also blown into the crawl space under the house at the rate of 1 pound to 1,000 sq. ft. of floor area. An electric duster with a 1-gallon-capacity hopper was used to apply the dust. In the attic the dust was applied entirely from the crawl hole, and under the house from one or two crawl holes or a larger number of foundation vents.

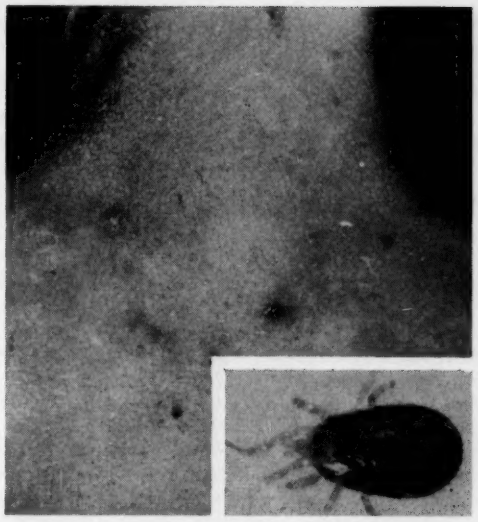

FIG. 1.—Back and neck of a woman, showing maculae caused by the bites of the rat mite. Inset at lower right, engorged mite.

Since in all cases the mites were already distributed throughout the dwelling, some dust was applied, by means of a small bellows hand duster, under the ticks of mattresses, on the spring supports of beds, along edges and in four corners of bed frames, into the junctures of the seats and the back rests or arm rests of sofas and lounges, as well as under the pillows, under furniture and other out-of-the-way places, and in a few places along the floor boards and ceiling moldings. This dust application was made in an effort to bring about immediate cessation of mite attacks. However, main reliance was placed on the dusting of the attic for long-term control.

In all buildings treated, severe infestations were experienced up to the date of treatment but ceased immediately after and were not resumed to the present date, except in one house. Here the housewife received a few more bites after treatment. It should be borne in mind that rat mites may live as long as 63 days with no food (Scott 1949), so those not coming into contact with the dust may continue to infest the inhabitants of the house. In this instance the housewife applied more dust behind the electric outlet plates and various other areas throughout the house not previously dusted, and no reinfestation has occurred. The periods since treatment of the six buildings range from 6 weeks to a year.

The silica aerogel does not depend on toxic action for its effect, but on the adsorption of the lipoid protective layer of the pest

[1] Accepted for publication January 26, 1960.

[2] The ammonium fluosilicate is added in the process of manufacture for certain advantages in industrial usage. In pest control the fluoride gives the silica particles a positive charge, improving deposit and adherence. It also increases the effectiveness of the dust against insects susceptible to fluorine. This powder, known as Dri-Die 67, was obtained for the experiments through the courtesy of Davison Chemical Company, Baltimore, Maryland.

that crawls over the residue. The removal of this lipid causes arthropods to die of dehydration. Since chemical decomposition is not a factor, it is reasonable to presume that one treatment of the attic of a house, and possibly the subfloor area also, may result in permanent control of the rat mite, even if the building continues to be infested with rats indefinitely. In none of the treated buildings was any effort made to control the rats after treatment for mites. The success of pest control operators with silica aerogel for the control of the rat mite in residences and apartment houses in southern California likewise tends to confirm this opinion.

In addition to mite control, the treatment should prevent infestation by drywood termites (Ebeling & Wagner 1959) and will kill cockroaches, silverfish, ants, fleas, and other household pests that crawl about in the attic or under the house or both (Tarshis 1959).

OTHER PARASITIC MITES CONTROLLED BY ATTIC DUSTING.—Besides the tropical rat mite, there are two species of bird mites and the house mouse mite (*Allodermanyssus sanguineus* (Hirst)) that may invade the home and attack man. The bird mites are the chicken mite, *Dermanyssus gallinae* (DeG.) and the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.). They may invade the home from bird nests in attics or under the eaves. The writer knows of two instances of successful attic dusting with silica aerogel dust by pest control operators when the inhabitants of houses were attacked by mites originating from bird nests in the attic. In one of these the dust contained 2% Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate), but would nevertheless have the long residual effectiveness attributable to the silica. Although the writer has no data on the control of the house mouse mite it may be assumed that control measures effective against rat mites and bird mites would also be effective against this species. The elimination of their host is, of course, also desirable.

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## Effect of Lime Applications to Soil on Japanese Beetle Larval Population[1]

J. B. POLIVKA, *Ohio Agricultural Experiment Station, Wooster*

In 1952, Wessel and Polivka reported that female Japanese beetles (*Popillia japonica* New.) deposit more eggs in soils of low pH values, and that the highest grub populations are found in those areas. These conclusions were obtained from a series of plots that were studied for only 2 years, 1949 and 1950. Since the original plots had to be abandoned at the end of the 1950 season, other plots were established at different locations in the State to obtain more data on the effect of different lime levels on the Japanese beetle larval population.

In a cemetery near Wellsville, Ohio, an area was found in 1950 that had been limed heavily about 1940. Upon checking the acidity of this area, it was found to have a pH of 7.0 whereas the unlimed portion of the same cemetery had a pH of only 4.3. Larval population surveys were begun in these areas in 1950 and continued each year through 1957. These data are shown graphically in figure 1.

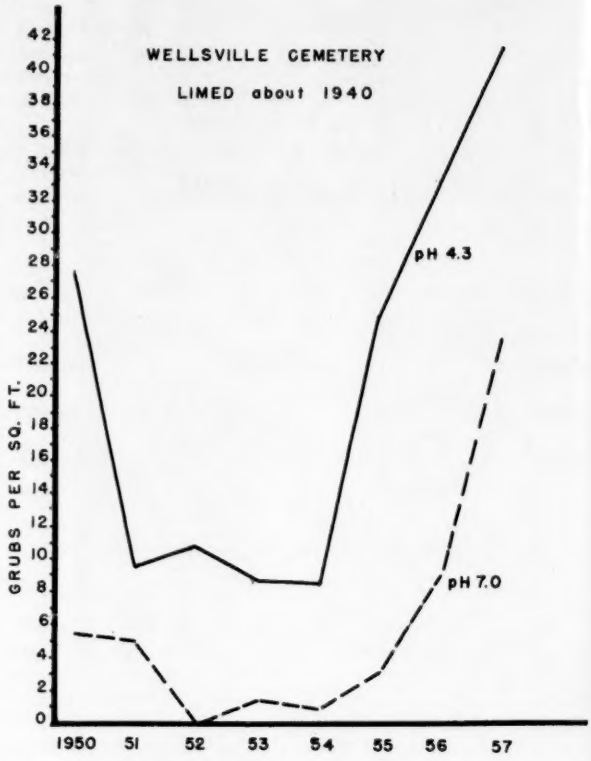


FIG. 1.—Japanese beetle grub population in relation to soil acidity. Wellsville Cemetery, Ohio.

Throughout the period of the study, the Japanese beetle grub population was from two to ten times greater in the acid soils than in the heavily limed area. The fluctuation in grub population from year to year in the two areas is considered to be due to differences in climatic conditions occurring during the different years, inasmuch as the two lines are nearly parallel.

In an experiment that was established in the Belleview Golf Course in Steubenville, Ohio, in April of 1954, hydrated lime was applied to a series of three plots at the rate of 1,500 pounds per acre. A similar number of plots were left untreated. These plots were surveyed for grub population in the fall of 1954, 1956, and 1957. Results of this study are shown graphically in figure 2.

It may be noted that a greater population was found in the unlimed area than in the limed section the first fall after application. A similar difference was found throughout the period of the study. The fluctuation in population from year to year is again credited to the variation in rainfall occurring during the different years. The pH values shown in figure 2 were obtained from soil samples taken from the plots in 1957.

In 1954 another series of lime plots was established on the Blackbrook Golf Course near Painesville, Ohio. In this experiment, hydrated lime was applied at the rate of 0, 1500, and 3000 pounds per acre. All treatments were replicated four times. The results obtained from these studies are shown in figure 3. The pH determinations were made in 1957, or 3 years after the lime was applied. It will be noted that the untreated plots had a pH of 5.7, the 1,500-pound rate a pH of 6.6, and the 3,000-pound rate

[1] Accepted for publication February 8, 1960.

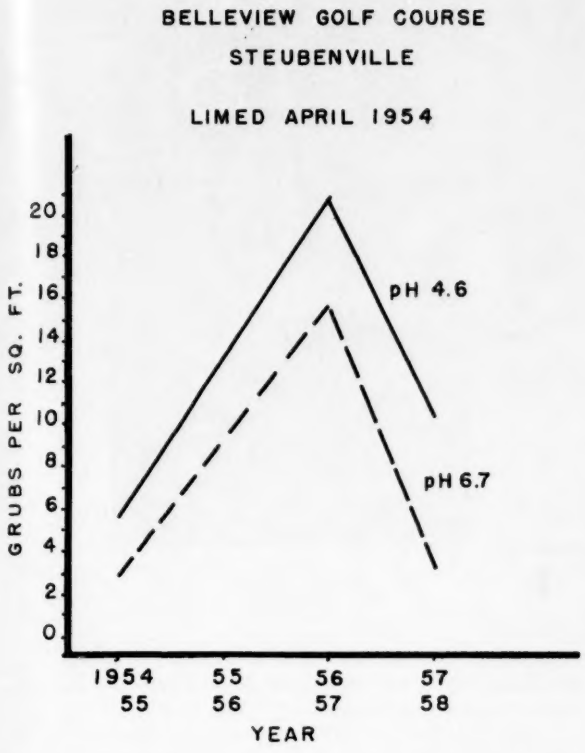


FIG. 2.—Japanese beetle grub population in relation to soil acidity. Belleview Golf Course, Steubenville, Ohio.

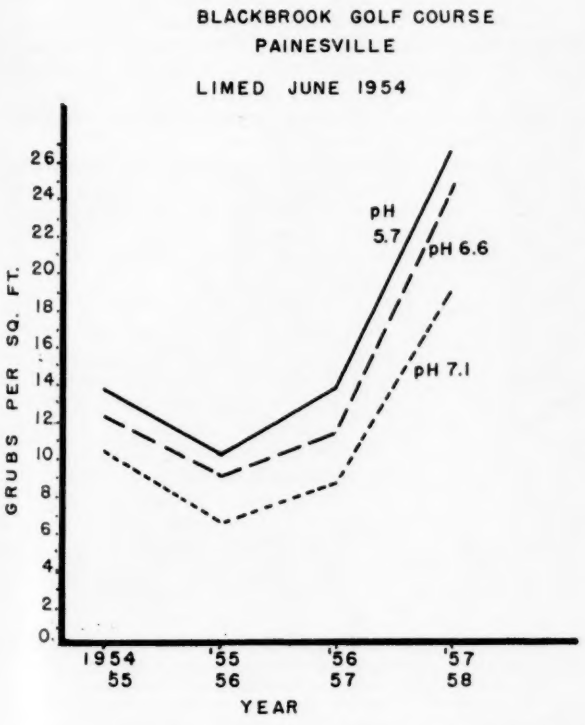


FIG. 3.—Japanese beetle grub population in relation to soil acidity. Blackbrook Golf Course, Painesville, Ohio.

a pH of 7.1. Again the data show that the greatest grub population was found in the untreated or low-pH soils and the lowest grub population in the high-pH soils.

That the Japanese beetle larval population is directly correlated with soil acidity seems to be definitely established. The studies also indicate that it is possible to obtain reduced levels of grub population by applying lime.

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## Residual Effectiveness of Certain Insecticides in Horn Fly Control[1]

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Several insecticides such as methoxychlor, malathion, Korlan® (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate), dimethoate, and Sevin® (1-napthyl *N*-methyl carbamate) give protection to cattle from horn flies, *Siphona irritans* (L.) when used as either sprays or dusts. Their use, however, has become a triplex problem of how long the insecticide will last, the most expedient method of application, and the possibility of insecticide contaminated milk or meat which in turn may be deleterious to man. The generalized use of the insecticides is therefore limited.

The tests reported herein were designed to study and compare the residual effectiveness of the insecticides mentioned (in protecting animals against horn flies) when used either as sprays or dusts. At this writing, there are two synthetic residual insecticides, methoxychlor and malathion, which have label approval for use on dairy cattle, when applied as dusts. Dust application is highly satisfactory under some situations, but somewhat impractical in others. Sprays are more practical than dusts in the conventional milking barn. In Maryland there is a gradual shift from the conventional milking barn to the milking parlor with stanchions for milking three or more cows at a time. In milking parlors, where studies were made during the summer of 1959, the use of dusts was found to be a practical procedure for applying insecticides to control horn flies.

PROCEDURE.—The 1959 tests in Maryland involved eight herds of cattle and five insecticides in one or more forms, replicated one or more times in some cases. The herds were treated with a single insecticide by spraying or by rubbing dust into each animal's hair. When the latter method was used dust was spread on the back, from shoulders to tail, and rubbed lightly into the hair with a stiff brush. When a spray was applied the entire animal was sprayed, exclusive of head and udder, with a compressed air sprayer. Each sprayed animal received approximately 1 pint of prepared spray. All sprays contained approximately 0.5% of the active ingredient. Dusts were applied so that all animals would receive approximately the same amount of active ingredient of a given insecticide. For example, 2 tablespoonsful of malathion 4% dusts were used to 1 tablespoonful of 8% Korlan or Sevin.

Fly counts were made prior to treatment and every second day thereafter until six inspections were made, or until the fly population built up to the pretreatment level. The counts before treatment exceeded 50 flies per animal, except in two cases, as indicated in table 1. The results were based on observations in the pasture where counts were made on eight animals in the herd, and averaged.

RESULTS AND DISCUSSION.—Malathion, Korlan, dimethoate and Sevin as used in these tests controlled horn flies either as

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**Table 1.—Residual effectiveness of insecticides for horn fly control.**

| Treatment | Average No. of Flies on 8 Cows (Days After Treatment) | | | | | | Rain Interference (Days After Treatment) |
|---|---|---|---|---|---|---|---|
| | 2nd | 4th | 6th | 8th | 10th | 12th | |
| *Sprays* | | | | | | | |
| Malathion | 2 | 5 | 5 | 25 | 50 | 50 | 7 |
| Malathion SF59[a] | 3 | 2 | 18 | 5 | 6 | 46 | |
| Sevin | 0 | 50 | 50 | — | — | — | 3 |
| Sevin | 18 | 35 | 50 | — | — | — | 3 |
| Sevin | 2 | 2 | 4 | 12 | 20 | 39 | 11 |
| Methoxychlor | 0 | 0 | 4 | 0 | 3 | 5 | |
| *Dusts* | | | | | | | |
| Malathion 4% | 0 | 3 | 3 | 14 | 20 | 45 | 9 |
| Malathion 4%[a] | 0 | 0 | 12 | 4 | 16 | 50 | 9 |
| Sevin 8% | 2 | 6 | 8 | 18 | 50 | — | 9 |
| Korlan 8% | 1 | 2 | 6 | 8 | 6 | 28 | 11 |
| Korlan 8% | — | 0 | 0 | 2 | 4 | 16 | 2 |
| Dimethoate 4% | 0 | 1 | 2 | 3 | 2 | 8 | 3 |
| Dimethoate 4% | 15 | 3 | 4 | 4 | 9 | 6 | 1 & 11 |

[a] Interpolated—pretreatment counts = 25 flies per animal; all other pretreatment counts = 50 flies per animal.

sprays or dusts. However, weather conditions, particularly rain, were found to be an important factor in residual effectiveness. None of the insecticides as used may be expected to protect animals for more than 2 weeks, whether used as a spray or dust. More frequently the period of protection was considerably less. The effectiveness of methoxychlor has been observed by the writers during 4 years. Whether used as a spray or dust, it has been found that residual effectiveness rarely exceeds 12 days under Maryland conditions. Excessive dosages gave little if any added residual effectiveness.

Of the dusts used in the tests described herein, Korlan and dimethoate showed distinct promise by controlling horn flies up to 12 days. Sevin, while not giving as long a period of control as the other dusts, has possibilities owing to its evident safety on dairy cattle. It is possible that Korlan and Sevin as dusts will give longer periods of control than these tests have indicated. These two insecticides were reformulated to 8% dusts by diluting with talc, which is alkaline in reaction. Since both of these chemicals are alkali sensitive a reduction of residual effectiveness might be expected. Malathion dust was somewhat less effective than Korlan and dimethoate. It was effective for periods of 8 to 10 days. Rain did not interfere with one of the malathion replicates. In this replicate there was little difference after 12 days between it and its counterpart in which rain occurred on the ninth day.

Both wettable powder and dust formulations appeared to absorb body oils and to cake on the hide and between the hairs. The degree to which this occurred seemed to influence the period of protection.

The sprays generally were not effective as long as dusts. One of the methoxychlor sprays in this series gave much longer protection than had been obtained in past years, perhaps because during the entire period of observation there was no rain. A new American Cyanamid Company formulation of malathion (SF 59) was observed to give protection 2 days longer than a standard emulsifiable concentrate formulation.

## An Unidentified Fungus Parasitic on the Seed-Corn Maggot[1]

F. E. STRONG, K. WELLS and J. W. APPLE[2]

In the course of an investigation of the seed-corn maggot, *Hylemya cilicrura* (Rondani) in Wisconsin, an unusual type of parasitism of adults was noted. The parasite, an internal fungus, has not been mentioned in North American literature. Smith (1927a, 1927b) in England described and illustrated a similar type of parasitism of the cabbage maggot, *H. brassica* Bouché.

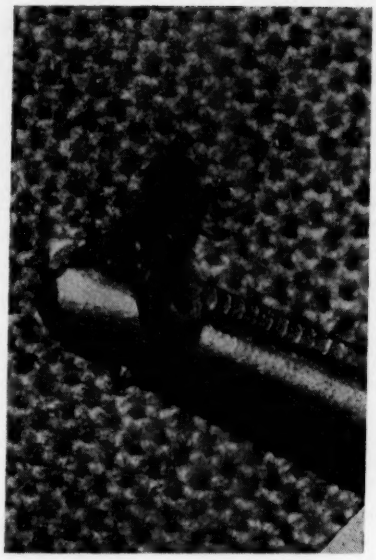

FIG. 1.—Hole caused by parasitic fungus in adult seed-corn maggot. ×5

Smith (1927b) believed the causative agent to be a fungus, probably belonging to the Entomophthoraceae, but was unable to make a positive identification.

A fly with parasitism of this type was collected on May 22, 1957 in Columbia County, Wisconsin. One week later, 30% of the flies captured were parasitized, and 2 weeks later, 47%. From June 25 to July 14, parasitized flies were difficult to collect. On July 16, however, 60% of trapped adults were parasitized. Very few parasitized flies were collected after August 5 but they were again collected in the same locality of Wisconsin in early 1958.

The diseased flies were readily characterized by the presence of a round hole situated on the midventral surface of the abdomen (fig. 1). The diameter of the hole was usually one-third to one-half the maximum width of the abdomen. Occasionally two smaller holes were present, one on each side of the ventral midline. Males and females were equally affected.

Although this fungus appeared to produce complete sterility, there was no perceivable difference between the behavior of parasitized and unparasitized individuals. Parasitized individuals appeared equally as active and fed as readily under caged conditions as unparasitized ones. No diseased females, however, were ever noted to deposit eggs.

Two diseased flies, preserved dry in cotton, were examined histologically. The abdomens were removed, dehydrated in a butyl alcohol series, and embedded in paraffin, as described by Johansen (1940). Cross-sections in series 10$\mu$ thick were stained with Safranin and Light Green and mounted in Diaphane. A section having two cavities is illustrated in figure 2.

Examination of the sections revealed that the cavities are lined with a closely packed, palisade-like layer of sporangiophores bearing uninucleate, obovate, sporangia[3], as shown in figure 3. Beneath the layer of sporangiophores are dense clusters of multinucleate hyphal bodies interspersed among the remaining viscera. These hyphal bodies are interwoven, apparently unbranched sections with rounded ends approximately 7.5 to

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Accepted for publication November 9, 1960.

[2] Departments of Entomology and Botany, University of California at Davis and Department of Entomology, University of Wisconsin at Madison, respectively.

[3] The terms "conidia" and "conidiophores" are frequently applied to these structures, but as Bessey (1950) and Moreau (1953) point out, they should be regarded as reduced sporangia.

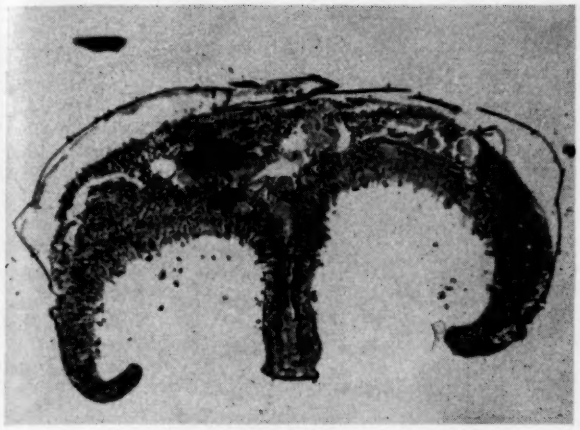

FIG. 2.—Cross-section of the abdomen of *Hylemya cilicrura* having two cysts caused by unidentified fungus. ×35.

17.5μ in diameter. Although it is difficult to trace the complete structures, it appears that the sporangiophores are rarely branched. The sporangia, which arise as the swollen tips of the sporangiophores, eventually become obovate and uninucleate. Basal septa are formed which delimit the sporangia. After release from the sporangiophores, the basal septa form the rounded bases of the sporangia. Released sporangia are always uninucleate and measure 21 to 27μ by 11 to 14μ. It is often possible to observe traces of the outer membrane which persist at the base of the released sporangia, similar to that described by Martin (1925) for *Conidiobolus villosus* Martin and as illustrated by Thaxter (1888) for certain species of *Entomophthora, e.g., E. aphidis* Hoffman.

The rounded bases of the sporangia differ from the basal papillae illustrated by Thaxter (1888) or by Hall & Dunn (1957) for certain species of *Entomophthora*. They also differ from those illustrated by Speare (1921) and Goldstein (1929) for *Massospora cicadina* Peck. The sporangia described here are more like those of *Entomophthora exitialis* Hall & Dunn (1957) and *E. aphidis* Hoffman as described by Thaxter (1888).

As the zygospores, azygospores, method of spore dispersal and germination of sporangia have not been observed, it does not seem justifiable to attempt to name this parasite at this time. It appears, however, as though the fungus is a member of the Entomophthoraceae, but examination of living material is necessary in order to substantiate this opinion.

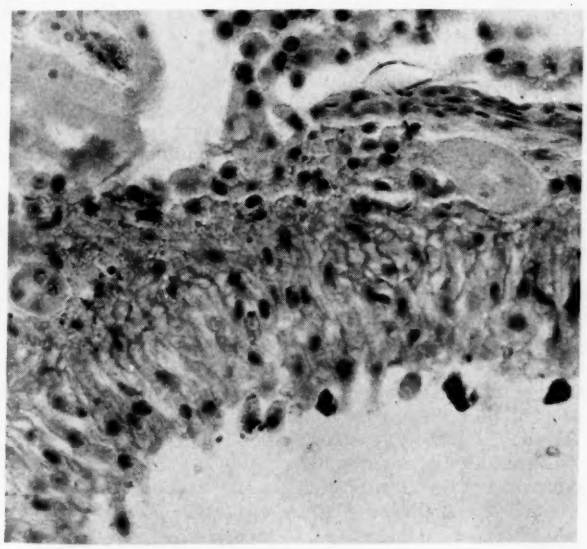

FIG. 3. Portion of cross-section of diseased abdomen. Note sporangiophores, and obovate, uninucleate sporangia, having rounded bases. ×192.

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## House Fly Pupation Under Baseboards[1]

JOSEPH L. MINKIN and HAROLD GEORGE SCOTT[2]

During October 1959, a house fly (*Musca domestica* L.) problem persisted in a guinea pig house at Chamblee, Georgia. Outdoor fly populations were low. Investigation revealed large numbers of house fly pupae under baseboards in the building (figs. 1 and 2). A quart of pupae, only a small portion of those present, were collected in a few minutes. By pressing the pupae to determine viability, approximately 20% of nonemerged pupae were found to be alive. Adult flies were observed emerging from pupae under the baseboards.

Reconstruction of the situation can be made from various observations: (1) adult flies deposited eggs in waste trays under the animal cages and the larvae underwent development there; (2) when mature, the larvae crawled down from the cages and across the floor in search of soil or other suitable pupation media; (3) upon reaching the floor without finding suitable media, they wriggled under the baseboards and pupated.

Routine weekly cleaning of cages had failed to control these flies because, at the regulated temperature (70°–72° F.) and generally high humidity, flies completed larval development in about 6 days, and pupal development in about 8 (West 1951, pp. 199–204). Pupal life may have been extended by the relatively unfavorable conditions.

Control under these circumstances probably could be achieved best by semiweekly cleaning of cages. Control was being maintained in adjacent buildings using 10% parathion-impregnated cord (30 linear feet per 100 square feet of floor) and residual sprays (5% DDT or 5% malathion-12.5% sugar at 1 gallon per 950 square feet).

This unusual site of house fly pupation might explain other cases of high indoor fly incidence in the absence of large outdoor

[1] Accepted for publication January 28, 1960.

[2] Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.

FIG. 1.—Baseboard removed to show deposit of fly pupae along wall.

populations. Also, it illustrates the tremendous survival potential of the house fly, and reemphasizes how difficult it is to control this species.

FIG. 2.—Closeup of fly pupae found under baseboard.

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## Laboratory Studies of Three-Lined Potato Beetle and Control with Various Insecticides[1]

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*University of Rhode Island, Kingston*

As a result of previous studies of the tolerance of the potato flea beetle *Epitrix cucumeris* (Harr.) to DDT, in 1956 dieldrin became the standard recommendation for its control in Rhode Island. Previously the three-lined potato beetle, *Lema trilineata* (Oliv.), was an occasional inhabitant of some potato fields, where it caused no damage. In 1957 the insect had become more plentiful, and by 1958 it was sufficiently abundant to cause comment and concern among potato producers. Accordingly in 1959, when severe damage to some fields occurred, an investigation was undertaken to determine the reasons for this increase in population. A preliminary report on the findings is given here.

The field and laboratory methods employed were similar to those reported previously by the authors Kerr & Olney (1959) in studies of the seasonal tolerance of the potato flea beetle to DDT. Adult three-lined potato beetles were obtained from a field located in an area where dieldrin had been used for three seasons. The insects were jarred into paper bags and within 1 hour of collection transferred in the laboratory to glass jars that had been treated previously with acetone solutions of DDT, dieldrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9,methano-2,4,3-benzodioxathiepin-3-oxide) or endrin, or were untreated. The inside of each jar and its aluminum-foil cover contained a total area of 200 square inches. Four dosage rates of each insecticide were used. In each case, actual deposits were

[1] Contribution No. 993 from the Rhode Island Agricultural Experiment Station, Kingston. Accepted for publication January 13, 1960.
[2] Entomologist and Agricultural Chemist, respectively.

found to vary only slightly from the desired values and all were determined chemically. The studies were conducted under room temperature conditions, similar to those existing out of doors, and of continuous light, both daylight and artificial. Four jars, each containing an average of 32 beetles, were used for each dosage rate. For each insecticide, except endrin, the test period was 48 hours. For endrin the test period was extended to 72 hours The dosage mortality data were plotted on logarithmic-probability paper, the lines fitted by eye and the LD-50 values interpolated or extrapolated, when necessary. The slope function of the regression lines was calculated according to the short method proposed by Litchfield (1949).

RESULTS.—Life-history studies showed two generations of the insect annually. Adults of the overwintered generation were present from late May to mid-July. Adults of the first generation began to appear in mid-July and oviposited beginning early in August. Appearance of second-generation adults was not ascertained. The laboratory studies reported here are concerned with overwintered adults and those of the first generation. Deposits of dieldrin as high as 360 micrograms per square inch failed to kill as much as 1% of the beetles of the overwintered generation, while those of the first generation were similarly unaffected by deposits as large as 281 micrograms per square inch. These two levels were the highest included in each test. The LD-50 value of DDT against overwintered adults was approximately 5 micrograms per square inch. Against first-generation adults it became progressively greater during late July to mid-August, with values of 22.5 micrograms per square inch on July 30, 115 on August 5, and 515 on August 12. In field applications for potato flea beetle control, the standard recommendation of three-eighths pound actual dieldrin per acre was found to be approximately 8 micrograms per square inch of potato foliage. When used in previously recommended schedules for the same purpose, 1 pound actual DDT per acre averaged 23 micrograms per square inch of foliage.

Thiodan was found to be considerably more effective at lower dosages than DDT. Here, too, there appeared to be a difference in the tolerance of the insect to this insecticide, for the LD-50 value in early July was 0.31 micrograms per square inch, while in mid-August it was 8 micrograms per square inch. Field application of three-fourths pound actual Thiodan per acre resulted in 2 micrograms per square inch of potato foliage immediately after application.

An investigation of endrin was undertaken against adults of the overwintered generation only, and somewhat erratic results were obtained. At deposit levels ranging from 5 to 37 micrograms per square inch, the material had no observable effect on the insects after exposure for 24 hours, only slight effect at 48 hours, and at 72 hours the greatest amount of kill at the highest dosage was 77%. In all cases the slope of the regression line was such that it was impossible to determine the LD-50 value.

From the data obtained it is evident that the sudden increase in three-lined potato beetle populations was owing to the ineffectiveness of dieldrin against the insect. Also, it appears that, while both DDT and Thiodan at the amounts ordinarily recommended would be effective against overwintered adults, such amounts might prove insufficient for adults of the first generation.

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## Sevin Residues on Soybeans Following Its Use for Armyworm Control[1]

L. J. HARDIN, W. W. STANLEY, D. E. GONZALEZ, and S. E. BENNETT,[2] *Tennessee Agricultural Experiment Station, Knoxville*

In August, 1959, following observed infestation of armyworm (*Pseudaletia unipuncta* (Haworth)) in a field of soybeans in East Tennessee, Sevin® (1-naphthyl methylcarbamate) was applied as wettable powder at three rates, 0.75, 1.5, and 3.0 pounds per acre, on respective replicated plots.

Foliage samples were collected from the treated and untreated plots immediately after the applications and again 4 days later, and analyses were made to determine the amount of Sevin residue present.

Counts of living larvae of the armyworm were made on four 1-square-foot areas of each replicated plot 1 day and again 4 days after treatment to determine the effectiveness of the various dosages applied.

RESIDUE ANALYSIS.—Immediately after the samples were received in the laboratory they were chopped into 1″ lengths, composited and "stripped" of residue by muddling 100-gram samples four successive times with methylene chloride, using 200, 150, 150, and 100 ml. each time, a total of 600 ml. The combined filtered extract of each sample was stored at −5° C. until analysis.

Aliquots of the extracts were analyzed by the revised p-nitrobenzene-diazonium fluoroborate colorimetric method developed by the Union Carbide Chemicals Corporation (Anon. 1958). By this method, the aliquot is evaporated under reflux and the solvent recovered. After dissolving in 5 ml. of acetone the residue is cleaned up by coagulation of impurities by means of ammonium chloride-phosphoric solution and filtration. The residue is equilibrated three times with methylene chloride and the aqueous phase discarded. After transfer of the solution from the separatory funnel into an Erlenmeyer flask, the methylene chloride is removed by evaporation as in the first step. The residue is then hydrolyzed with methanolic KOH and the color developed in glacial acetic acid by means of the fluoroborate reagent. Absorbance is read at 475 mμ and the amount of Sevin determined by comparison of the absorbance with that of a standard curve prepared concurrently.

The results of analyses for residues of Sevin and of counts of armyworm larvae are shown in table 1.

DISCUSSION OF RESULTS.—After 24 hours, the reduction in the number of larvae was about 50% for each of the three dosages applied. After 96 hours, the 0.75- and 1.50-pound applications were very effective, whereas the 3-pound dosage resulted in 99% reduction in live larvae. The amount of Sevin residue on the crop immediately after application was 24, 78, and 125 p.p.m., respectively. After 96 hours, the residues for the 0.75- and 1.5-pound dosages were 2 and 4 p.p.m., respectively, whereas for the 3-pound dosage the residue was 30 p.p.m.

Although no tolerance has been established for Sevin on soybeans, a tolerance of 10 p.p.m. has been established for beans. These results indicate that application of 1.5 pound per acre of Sevin (wettable powder) would result in residues of less than 10 p.p.m. after 4 days and give about 90% control of armyworm larvae. Practically complete kill of the larvae resulted from the 3-pound application, but a longer period would be required to have the residue reach the 10-p.p.m. level. The present results, and unpublished data obtained on other forage crops, indicate that residues from the 3-pound dosage of Sevin would be below the 10 p.p.m.-level about 7 days after application if the rate of disappearance continued the same as for the first 4 days.

[1] Accepted for publication January 25, 1960.

[2] Associate Chemist, Entomologist, Assistant in Chemistry, and Assistant in Entomology, respectively.

Table 1.—Sevin residue on soybeans and live larvae of the armyworm present.

| Dosage (Lb./A.) | Net Sevin Residue (p.p.m.)[a] | | Live Larvae After[b] | | | |
|---|---|---|---|---|---|---|
| | | | 24 Hours | | 96 Hours | |
| | Initial | 96 Hours after Application | Total | Per Sq. Ft. | Total | Per Sq. Ft. |
| Untreated | 0.6 | 0.8 | 170 | 8.5 | 192 | 9.6 |
| 0.75 | 24.0 | 2.0 | 85±10 | 4.3 | 25±2.3 | 1.3 |
| 1.5 | 78.0 | 4.0 | 106± 3.5 | 5.3 | 22±3.6 | 1.2 |
| 3.0 | 125.0 | 30.0 | 80± 4.5 | 4.0 | 2± .03 | 0.1 |

[a] Average of residue determinations made in duplicate. S. D. of duplicate determinations, 0.4.
[b] Average of four replicated plots.


REFERENCE CITED

*Anonymous.* 1958. Union Carbide Chemical Corporation Tech. Pamphlet 1543–16.


## Pickleworm Control and Residue Studies with Malathion and Phosdrin[1]

JAMES E. ROBERTS and LEO E. ANDERSON[2]

The pickleworm *Diaphania nitidalis* (Stoll) is the most important pest of cucumbers and other cucurbits in Georgia. According to Dupree *et al.* (1955) this insect can be controlled by proper applications of DDT, lindane, parathion, methyl parathion, or EPN. Regular DDT caused more plant injury than purified DDT. Malathion was tested only 1 year and gave fair results. However, the rate of application of malathion was considered to be somewhat low. Dupree stated that a minimum of six applications was usually required for good control. He further stated that these applications were most effective when started at the time of bloom and continued at 5- to 7-day intervals.

Cucumbers are usually harvested at 2- or 3-day intervals. In view of the residue tolerances set by the Food and Drug Administration (FDA) and the repeated insecticidal applications necessary for pickleworm control, use of an insecticide with a short residual life is very important. Hence, tests were conducted in 1959 to compare the effectiveness of malathion and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) for pickleworm control and to determine the rate of disappearance of these two insecticides on cucumbers.

PROCEDURE.—The Model variety of cucumbers, a popular pickling variety, was planted for this study on May 4. Each plot was eight rows wide and 30 feet long and the hills were spaced 4 feet apart. Treatments were replicated four times in a randomized block design. Four applications of each insecticide were made at 5- to 7-day intervals, beginning June 25 and ending July 24. Malathion was applied as a 4% dust at the rate of 1.5 pounds actual per acre. Phosdrin was applied as a 2% dust at the rate of 0.5 pound actual toxicant per acre.

The following procedure was used to evaluate pickleworm damage. Cucumbers were harvested nine times at 2- and 3-day intervals, beginning July 1 and ending July 20. The entire yield from each plot was examined, and the numbers of worm-damaged cucumbers were recorded.

Subsamples of cucumbers were taken randomly from entire plot yields for insecticide residue analyses. These subsamples were placed in a freezer after examination for pickleworm damage and held until preparation for extraction. The extraction procedure was as follows: Unwashed cucumbers were sliced and macerated in a Waring Blendor. A 250-gram sample of the macerated material was weighed into a wide-mouth 1-gallon glass jar along with 500 milliliters of the recommended solvent. Carbon tetrachloride was used for extraction of malathion, and chloroform was used for extraction of Phosdrin. The wide-mouth jars were sealed and tumbled for 1 hour in a tumbling machine as described by Gunther & Blinn (1955). After tumbling, samples were filtered through sharkskin filter paper with the aid of 5 to 10 grams of Hyflo-Super-Cel, which was placed on the paper. After filtering, 100 grams of sodium sulfate were added to each sample to remove water and the extract solutions were refiltered and held in a freezer until analyses were made.

Table 1.—Effectiveness of two insecticides for control of pickleworm on cucumbers. Experiment, Georgia, 1959.

| Harvest Date (July) | Average Number of Worm-Damaged Cucumbers[a] | | | L.S.D. at 1% |
|---|---|---|---|---|
| | Malathion 1.5 lb. Actual/A. | Phosdrin 0.5 lb. Actual/A. | Untreated | |
| July 1 | 0.97 | 0.71 | 0.83 | n.s. |
| 3 | 0.71 | 0.71 | 1.14 | n.s. |
| 6 | 0.84 | 0.71 | 2.47 | 1.56 |
| 9 | 1.81 | 1.41 | 3.18 | 1.35 |
| 11 | 0.93 | 0.71 | 3.15 | 1.76[b] |
| 13 | 1.06 | 0.93 | 2.52 | 1.36 |
| 15 | 1.35 | 0.91 | 2.95 | 1.04 |
| 17 | 0.71 | 0.71 | 1.32 | n.s. |
| 20 | 0.71 | 0.84 | 1.06 | n.s. |
| Totals | 2.49 | 1.77 | 6.52 | 2.37 |

[a] All data were transformed by use of formula $\sqrt{N+0.5}$.
[b] L. S. D. at 5% level. Not significant at 1%.

Malathion residue were determined by the modified colorimetric method of Conroy (1957). Phosdrin residues were determined by an enzyme inhibition-spectrophotometric method (Anonymous 1956).

RESULTS.—As shown in table 1, total numbers of worm-damaged cucumbers in malathion- and Phosdrin-treated plots were significantly less than in untreated check plots at the 1% level. Analyses of data on worm-damaged cucumbers by harvest dates showed that both insecticides significantly reduced damage on five of the nine dates, four of which were highly significant.

Results of Phosdrin and malathion residue determinations are presented in table 2. According to these determinations, Phosdrin residues were below the FDA tolerance of 0.25 p.p.m. 2½ hours after treatment. Recovery of Phosdrin added to extract solutions averaged 99%.

No malathion residues were found on cucumbers 2½ hours after application. Recovery of malathion added to extract solutions averaged 95%.


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[1] Paper No. 372, Journal Series, Georgia Experiment Station, Experiment, Georgia. Accepted for publication February 2, 1960.
[2] Assistant entomologist and assistant chemist, respectively, of the Georgia Experiment Station, Experiment, Georgia.

**Table 2.—Phosdrin and malathion residues on cucumbers at various intervals after treatment. Experiment, Georgia, 1959.**

| TREATMENT TO HARVEST (HOURS) | RESIDUES (P.P.M.) | |
|---|---|---|
| | Malathion | Phosdrin |
| 2.5 | nf[a] | 0.097 |
| 24 | nf | .073 |
| 72 | nf | .000 |

[a] nf = less than 0.5 p.p.m.

hibition spectrophotometric method (tentative) Shell Chemical Corporation, Agricultural Chemical Sales Division, New York 22, New York.

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## Apparatus for Insecticide Assay[1]

CHARLES C. HASSETT,[2] RAYMOND KIRK,[2] and GEORGE B. CRAIG

Negative photomigration is the basis of the test described by Burchfield *et al.* (1952) and Burchfield & Hartzell (1955) for the purpose of assaying the effects of insecticides on larvae of *Aedes aegypti*. This physiological test has proved to be simple, rapid, and useful. It practically eliminates guesswork in the compilation of mortality data. The use of the test in this laboratory has resulted in modifications of the apparatus to a somewhat simpler form which can be constructed readily in any laboratory shop.

The essential parts of the apparatus are the trough, screen gate, and lamp. The trough is made from Lucite, with sides $12\times3\times\frac{1}{8}$ in. and ends $3\times3\times3\times\frac{1}{8}$ in. This trough is mounted on a base which can be of plastic or wood, shown in figure 1. A block is placed at the end of the baseboard to insure correct positioning of the trough.

A standard 40-watt lamp is mounted in a porcelain socket at the end of the baseboard, with a space of 2 inches between it and the end of the trough. A metal shield covers the lamp and provides anchorage for a toggle switch and the arms on which are mounted the screen gate. This gate is made of 24-mesh brass screening with a narrow edging of solder, carefully fitted to the trough. It is set 2.5 inches from the end of the trough adjacent to the lamp.

In performing an assay, the chosen concentrations of insecticide are prepared and 250-ml. aliquots are put into 1-pint Mason jars. Twenty larvae are added to each jar. After standing 24 hours at 25° C., the contents of a jar are poured into the trough behind the gate. When the solution levels off the gate is raised and the light turned on. Larvae that do not succeed in swimming past a mark half-way along the trough in 1 minute are counted as dead.

Lucite is known to absorb insecticides, but in a 1-minute test this factor will not be significant. For the same reason, heating of the water is negligible and no heat filter is provided. A possible further improvement can be added by arranging the light switch so that raising the gate turns on the light.

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[1] Accepted for publication February 8, 1960.

[2] Physiology Division, U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Maryland.

[3] Department of Biology, University of Notre Dame, South Bend, Indiana

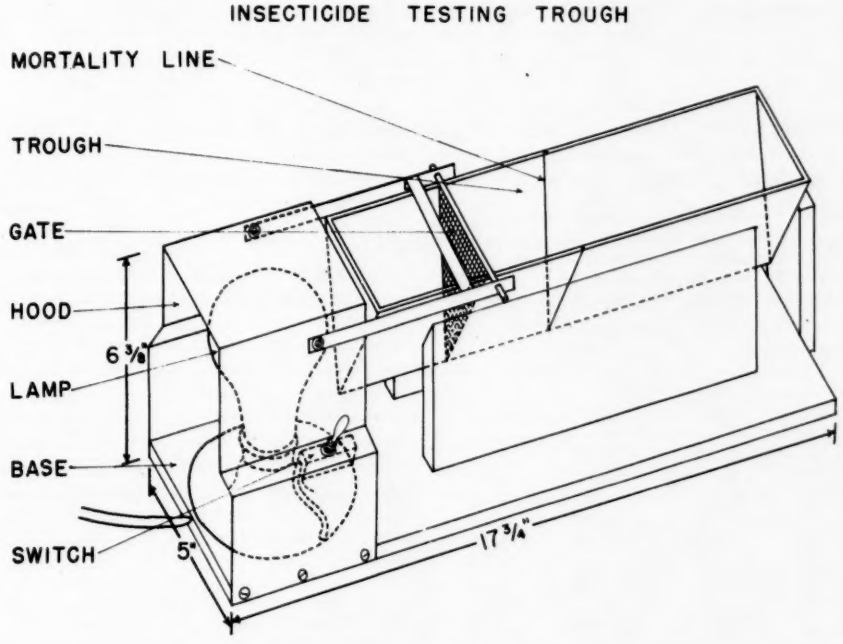


FIG. 1.—Trough and light source for bioassay of insecticides.

## Residues of Sevin and 1-Naphthol on Forage from Aerial Applications of Sevin in Oil[1]

E. W. HUDDLESTON and GEORGE G. GYRISCO[2]
*Cornell University, Ithaca, New York*

Since Sevin® (1-naphthyl methylcarbamate) showed promise as a replacement for DDT in early laboratory and field tests conducted against the gypsy moth by the Plant Pest Control Division of the U. S. Department of Agriculture in 1958, Cornell University was asked to undertake a further study concerned with the residues remaining on forage crops following aerial application of 1 gallon of a Sevin-kerosene mixture (1 pound of Sevin) per acre. While the actual study was near Fulton, outside of any known gypsy moth infestation, conditions were chosen to correspond as closely as possible to those that would be found under conditions of actual large-scale aerial treatment of infested areas.

MATERIALS AND METHODS.—One-acre plots were delimited in each of two large fields, one predominately red clover and the other predominately grass with some red clover. On the evening of July 30, 1958, the plots were sprayed by a 135 Piper Cub. Although wind was calm at this time and drift was at a minimum, extra spray runs were made on each side of the plots to assure complete coverage. Check or untreated samples were taken at 10 locations within each plot just prior to application. Immediately after spraying and 1, 3, 5, 8, 14, 21, and 49 days after application, additional samples from each plot were taken and immediately frozen. Each total sample consisted of all the foliage cut from within a 1-square-foot frame placed over the foliage at 10 systematically chosen locations, five in each half of a plot. After freezing, the samples were packed with dry ice and shipped by air express to South Charleston, West Virginia, for chemical analysis. The samples were analyzed by the Quality Control Laboratory, Union Carbide Chemicals Company by their method 30-UIA15-7 and are reported as parts per million of Sevin and 1-naphthol on a green weight basis (table 1).

DISCUSSION AND RESULTS.—The level of sensitivity in the Sevin analysis was 0.20 p.p.m., while the sensitivity for the 1-naphthol analysis was 0.23 p.p.m. When corrected for the check values, the level of Sevin was found to be below the sensitivity of the method 21 days after application. Although values of 0.01 and 0.12 are shown in table 1 for 1-naphthol, no 1-naphthol could be detected as these values are all below the sensitivity of the method.

These data indicate a rapid loss of Sevin from aerial application of 1 pound of Sevin in 1 gallon of a Sevin-kerosene mixture per acre to a forage crop. No 1-naphthol could be detected, either because little or none was formed on plants in the field or because 1-naphthol was lost rapidly from treated foliage as it was formed.

In contrast to DDT residues (Huddleston 1958) Sevin-in-oil residues on forage are neither as great nor persist as long.

**Table 1. Residues of Sevin and 1-naphthol remaining on legume-grass foliage for various intervals following aerial application of a Sevin-kerosene mixture at the rate of 1 pound of Sevin per acre. Fulton, New York. 1958.**

| Days After Application | Residue in p.p.m. Remaining on Foliage[a] | | | |
|---|---|---|---|---|
| | Sevin | | 1-Naphthol | |
| | Rep I | Rep II | Rep I | Rep II |
| 0 | 34.67 | 30.37 | 0.02 | 0.01 |
| 1 | 15.87 | 13.67 | 0.03 | 0.05 |
| 3 | 14.47 | 13.57 | 0.02 | 0.02 |
| 5 | 10.27 | 4.27 | 0.12 | 0.00 |
| 8 | 1.97 | 1.97 | 0.00 | 0.00 |
| 14 | 0.30 | 0.97 | 0.00 | 0.00 |
| 21 | 0.09 | 0.23 | 0.00 | 0.01 |
| 28 | 0.10 | 0.10 | 0.00 | 0.00 |
| 49 | 0.09 | 0.08 | 0.00 | 0.00 |

[a] Uncorrected for check; Sevin check = 0.20; 1-Naphthol check = 0.23.

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[1] The work reported here was partly supported by funds from Regional Project NE-36. Accepted for publication February 15, 1960.

[2] The authors are indebted to Union Carbide Chemicals Company, the Plant Pest Control Division of the U. S. Department of Agriculture and R. L. Ridgway and S. W. Jacklin of Cornell University for participation in certain phases of the work.

## Toxicity of Cellulose Acetate Sheeting to Leguminous Plants

ROBERT W. KIECKHEFER and JOHN T. MEDLER[1]

While conducting experiments on the biology of the potato leafhopper, *Empoasca fabae* (Harris), in the greenhouse during the spring of 1959, it was noted that the cellulose acetate cages used to confine the leafhoppers on host plants were having an adverse effect on the plants. Dark lesions appeared on the leaves of broad bean plants (*Vicia faba* L.) within 2 days after being placed in such cages and most plants died after a week's exposure. Alfalfa plants (*Medicago sativa* L.) yellowed severely in the cages and succumbed within 2 weeks.

In the 1930's F. W. Poos[2] experienced considerable trouble with acetate base plastic cages while conducting research on potato leafhopper injury and boron deficiency in alfalfa at Arlington, Virginia. He corrected his problem by using nitrate base plastic.

Maramorosch (Science 115: 263, 1952) reported on similar symptoms in crimson clover (*Trifolium incarnatum* L.) under cellulose acetate cages. By means of spectrophotometric analysis and bioassay he identified the toxic constituent as diethyl phthalate. This chemical is generally employed as the plasticizer in the manufacture of cellulose acetate sheets.

As most of the plastic sheeting commercially available is acetate base, a special inquiry was made to locate a supply of plastic suitable for our greenhouse research. A vinyl plastic was obtained that proved to be satisfactory in subsequent greenhouse tests.[3]

A check on the toxicity of the two types of plastic was made according to the goldfish bioassay method of Maramorosch. Goldfish, approximately 3 cm. in length, were exposed to 2- by 2-inch squares of cellulose acetate or vinyl sheeting in 3000 cc. of water. The squares of plastic weighed approximately 1.2 grams. In four replications, fish exposed to cellulose acetate died within 3 days after initiation of the test while those exposed to vinyl plastic, and the fish used as controls, were alive and healthy when the experiment was terminated after 10 days.

A colorimetric chemical test was performed which indicated the presence of a phthalate in the cellulose acetate plastic sheeting, but none in the vinyl. About 2.5 gm. of the plastic sheeting was cut into small pieces and immersed in 10 cc. of xylene for 24 hours. The xylene extract was then heated to 100° C. in a water bath and held at that temperature for about 15 minutes after which 0.5 cc. of concentrated $H_2SO_4$ and 0.5 cc. of phenol were added. While the solution was cooling, enough KOH was added to neutralize it. Development and retention of a distinct pink color (phenolphthalein) in the solution indicated the presence of a phthalate.

[1] Research Assistant and Associate Professor, respectively. Department of Entomology, University of Wisconsin, Madison. A contribution from North Central Regional Project NC-29. Accepted for publication March 7, 1960.

[2] Personal communication.

[3] Cadillac Plastic Co., Detroit, Michigan.

# OBITUARY

**Emilio Viale**
**1921-1959**

Emilio Viale suffered a sudden heart attack at his home in Wilmington, Delaware, on the evening of December 23, 1959, and passed away en route to the hospital.

Emilio, or Vi as his many friends called him, was born in Genoa, Italy, on April 18, 1921. He was reared, however, from the age of five in Lima, Peru, and attended elementary schools in that city. In 1944 he was graduated from the National Agricultural College of La Molina, also located in Lima. Upon graduation, he worked for a year as an entomologist in the Peruvian Ministry of Agriculture and for the same period as head of the malaria control unit at the experiment station in Tingo Maria, Peru. Vi then entered Kansas State College, Manhattan, Kansas, as a graduate student and Research Assistant, from which he received the Ph.D degree in entomology in 1950. He always had a wide interest in natural history, and particularly in insect life; and during a vacation period while at Kansas State, he, along with a few other students, completed an insect collecting trip by jeep all the way from the United States to Ecuador.

After leaving Kansas State, Vi accepted a position at the Interamerican Institute of Agricultural Sciences, Turrialba, Costa Rica, as the first entomologist to be assigned to the staff. During the 3 years spent at Turrialba, Vi not only worked on several insect pest problems of concern to Latin American crop production, but also served as an entomological consultant for several coffee growers' associations. In addition, he taught entomology courses to students working towards Masters degrees, and was one of the favorite teachers and advisers of students while at the Institute.

In 1953, Dr. Viale joined Hercules Powder Company, for whom he worked until his death. He was first assigned as a technical representative in the Company's Agricultural Chemicals Division and was stationed in Sao Paulo, Brazil. During the next 5 years, he traveled extensively in South America in the interests of technical sales of the Company's existing products, as well as in the development of new products. In 1958, Vi was placed in charge of field development of new agricultural chemicals and was transferred to Hercules' Home Office in Wilmington, Delaware.

In the summer of 1954, upon a visit to the United States, Vi was married to Anna Marie Dye of Arlington, Virginia. The couple first met in Turrialba, Costa Rica, where Anna Marie had been assigned to the Institute Staff, after first having worked for the Pan American Union in Washington, D. C. The newlyweds lived in Sao Paulo, Brazil, until moving to Wilmington.

Vi was a member of the Roman Catholic Church and the following scientific societies: Sigma Xi, Gamma Sigma Delta, Kansas Entomological Society, and the Entomological Society of America.

In addition to his wife, Dr. Viale is survived by his mother, Mrs. Rosa Carbone de Viale; a sister, Margarita Viale de Sanguinetti; two brothers, Rodolfo and Fausto, all residing in Lima, Peru.

To those who knew Vi, it was a simple matter to understand why he had so many friends. He was wonderfully sympathetic to everyone's problems and possessed an understanding and warmth of personality that seemed to create an immediate bond of friendship between himself and those he met. He had a high degree of integrity in his everyday affairs, as well as a great deal of intellectual honesty in his business and scientific pursuits. His passing will be sorely felt by his associates and by his host of friends all over the world.

H. F. PIERCE

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